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Comparison of High-Performance Liquid Chromatography and Potentiometric Titration Methods for the Content Determination of Metronidazole API

Derouicha MATMOUR[a,b,c,e,*], Khalil Fateh Eddine HASSAM[d], Nadjib HAMOUM[d], Yassine MERAD[b], Nassima HAMDI ZIANI[d], Houari TOUMI[c]

[a] *Therapeutic and Pharmaceutical Chemistry Laboratory, Pharmacy Department, Faculty of Medecine, University of Sidi Bel-Abbes, Sidi Bel-Abbes, 22000, Algeria*
[b] *Central Laboratory, University Hospital Center of Sidi Bel-Abbes, Sidi Bel-Abbes, 22000, Algeria*
[c] *Research in Pharmaceutical Development Laboratory, Pharmacovigilance Department, University Hospital Center 1st November, Faculty of Medecine, Oran 1 University, Oran, 31000, Algeria*
[d] *Quality control laboratory, WanyLab, Algiers, 16002, Algeria*
[e] *Saidal Team Pharmaceuticals Industries, Medea, 2600, Algeria*

Abstract

This study describes a comparative evaluation of High Performance Liquid Chromatography (HPLC) and potentiometric titration methods for the content determination of six samples of *Metronidazole* API, collected from six pharmaceutical industries installed in Algeria. For the content determination by HPLC, a liquid chromatography apparatus HPLC-UV device (Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC systems, Germany) equipped with an automatic injector and UV/Vis detector was used, the chromatographic conditions were regled at temperature: 25 °C, flow rate: 1 mL/min, injection volume: 10 µl, Column: C_{18} (5 µm x 4.6 mm x 250 mm) and the wavelength λ: 315 nm. For the content determination by potentiometric titration, a potentiometer brand METTLER TOLEDO DL_{50}, a titrant solution of perchloric acid 0.1M and potassium phthalate acid standard solution 0.1 M were used. All samples had a content meeting the required standard. The evaluated methods showed to be adequate to quantify Metronidazole in the pharmaceutical raw material. The potentiometric titration didn't required the reference substance use than HPLC method but the manipulation was rapid and the margin error was important than of HPLC method.

Keywords:
Metronidazole, Pharmaceutical raw material, Content determination, Dosage, HPLC, Potentiometric titration.

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*Corresponding author
Email address: drmatmour24@hotmail.fr (Derouicha MATMOUR)

1. Introduction

The quality of medicines remains a major determinant of the success of any health system [1] [2] . Poor quality medicines can take several forms: total absence of active substances, incorrect dose of active substances, and presence of toxic derivatives in the drug composition and poor dissolution profile [3]. In the drug development and pharmaceutical control, chemical analysis plays a key role to ensure a high efficacy and safety for patients [4]. For this reason, appropriate methods of quality control are of paramount importance to the pharmaceutical industry [5]. Thus, the pharmaceutical quality control should ensure use of appropriate analytical methods, of which it is observed a trend to utilize faster and more efficient techniques with cost savings and reduction in solvent consumption [6].

Metronidazole (**Figure 1)** is an antibacterial antiparasitic drug, belonging to the nitro-5-imidazole family [7]. It is used in the treatment of acne rosacea, hepatic amoebiasis, intestinal amoebiasis, giardiasis, anaerobic germ infections, urogenital trichomoniasis, and vaginitis [8].

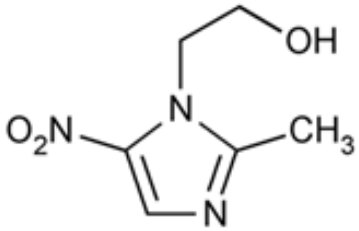

Figure 1: Chemical structure of *Metronidazole* [9]

In Algeria, *Metronidazole* is produced by more than 20 pharmaceutical producers [10]. This study describes a comparative evaluation of High Performance Liquid Chromatography (HPLC) and potentiometric titration methods for the content determination of six samples of *Metronidazole* Actif Pharmaceutical Ingredient (API), collected from six pharmaceutical industries installed in Algeria.

2. Materials and methods

2.1. Collection of samples

Six samples of *Metronidazole* API were collected from six pharmaceutical industries installed in Algeria by referring to the Algerian drug nomenclature dated the 31st December 2014 [10]. The samples are collected during the period from 1st April 2015 to 31st December 2016. The compendium covers not only the API but also the following necessary information (origin, supplier/manufacturer, expiration date, analysis certificate, synthesis route, Drug Master File, etc. [11]. The samples were not expired and we labeled them as follows: M1, M2, M3, M4,

M5 and M6. They were stored at room temperature, protected from light and humidity and analyzed prior to their expiration date. For some samples, we did not receive all the necessary informations.

2.2. Content determination by HPLC

A liquid chromatography apparatus HPLC-UV device (Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC systems, Germany), equipped with an automatic injector and UV/Vis detector was used, the chromatographic conditions were regled at temperature: 25 °C, flow rate: 1 mL/min, injection volume: 10 µl, Column: C_{18} (5 µm x 4.6 mm x 250 mm) and the wavelength λ: 315 nm [12] [13]. The mobile phase was composed of 30 volumes of methanol mixed with 70 volumes of monopotassium phosphate solution at 1.36 g/L.

The standard solution and the test solutions were prepared at 0.1 mg/mL [12].

The *Metronidazole* SCR purchased from the European pharmacopeia (Eur Ph) (Strasbourg, France), was used as reference standard for the assay [12].

2.3. Content determination by potentiometric titration

A potentiometer brand METTLER TOLEDO DL_{50}, a titrant solution of perchloric acid 0.1 M and potassium phthalate acid standard solution 0.1 M, were used. The test solutions were prepared at 3 mg/mL in anhydrous acetic acid [14], [15].

3. Results and discussion

3.1. Content determination by HPLC

The **Figure 2** and **Figure 3** show respectively the obtained chromatograms with the standard solutions. The **Figure 4 , 5, 6, 7, 8** and **9** show respectively the obtained chromatograms with the test solutions of M1, M2, M3, M4, M5 and M6 samples. **The Table 1** summarizes the content results of *Metronidazole* API "As it is" and in "Anhydrous substance" by HPLC of the various samples of *Metronidazole* analyzed as well as the standards required by the Eur Ph and the United States Pharmacopeia (USP).

According to the four injections of weight 1 standard and the two injections of weight 2 verification standard, the *Metronidazole* peak was detected and its retention time was around 6 min, a value close to that required by the Eur Ph and USP [12]–[16]. The coefficient of variation between the four injections was 1.08%; it was less than 2% , which confirms that this analysis method is reliable.

According to the Eur Ph and USP standards [12]–[16], the content of *Metronidazole* in "Anhydrous substance" must be between 99.0% and 101.0% . All M1, M2, M3, M4, M5 and M6 samples had an anhydrous *Metronidazole* content meeting the standards (**Table 1**).

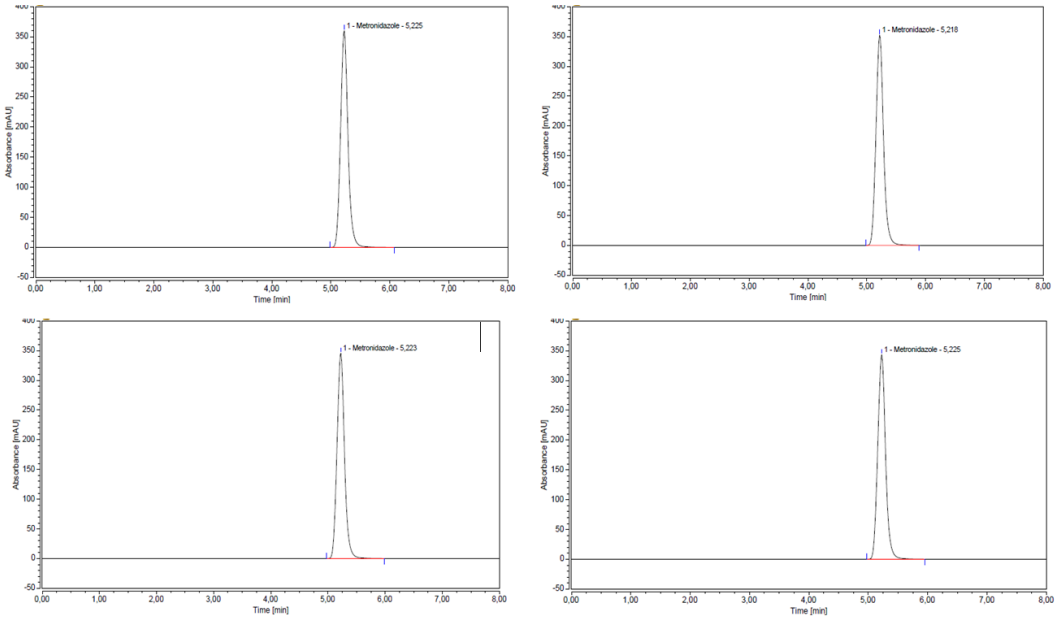

Figure 2: Chromatograms of standard solution weight 1 (4 injections)

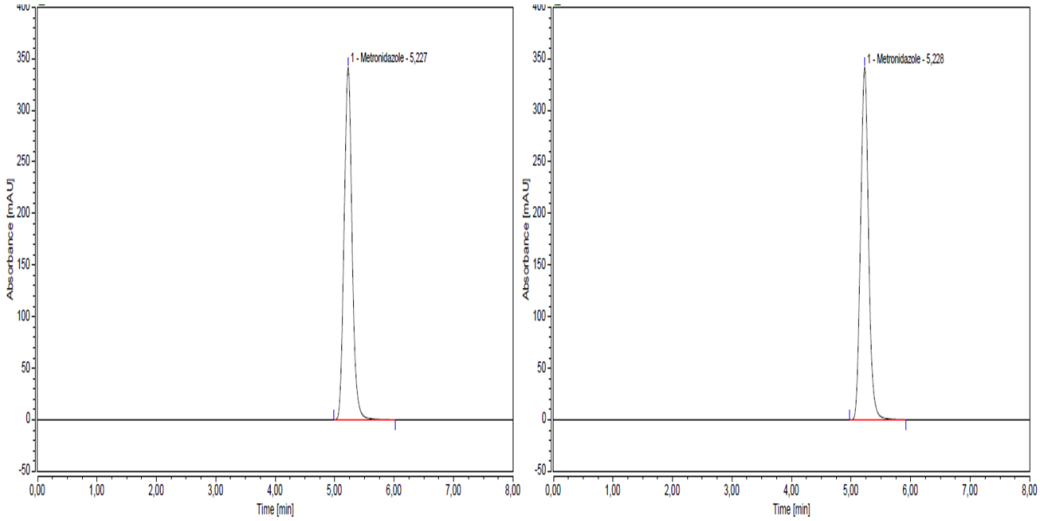

Figure 3: Chromatograms of standard solution weight 2 (2 injections))

3.2. Content determination by potentiometric titration

The **Figure 10** shows the reaction involved between *Metronidazole* and perchloric acid. The **Figure 11** shows respectively the graphics of potentiometric titration of M1, M2, M3, M4, M5

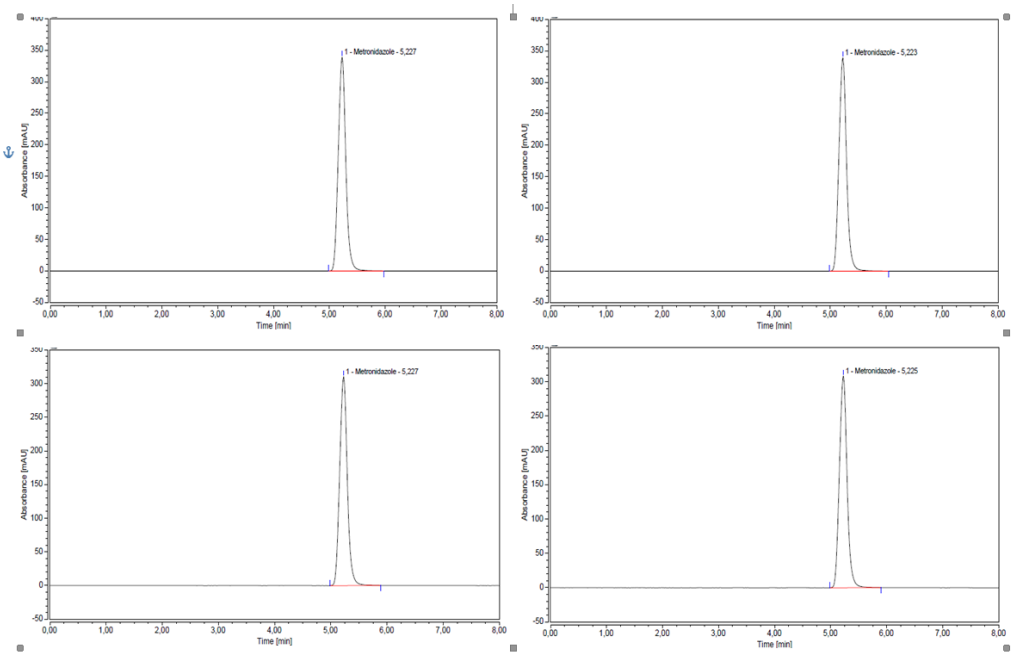

Figure 4: Chromatograms of M1 sample (4 injections)

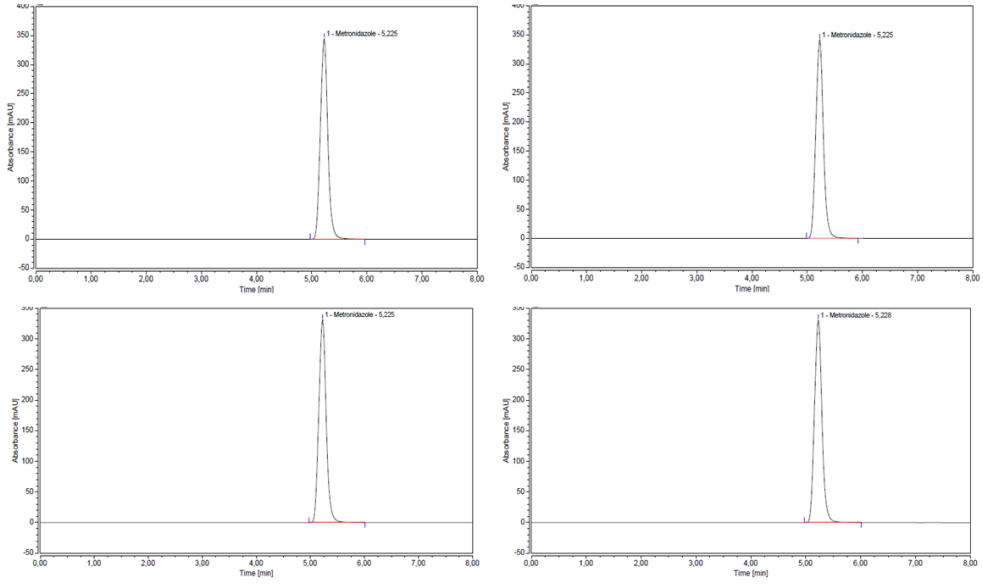

Figure 5: Chromatograms of M2 sample (4 injections)

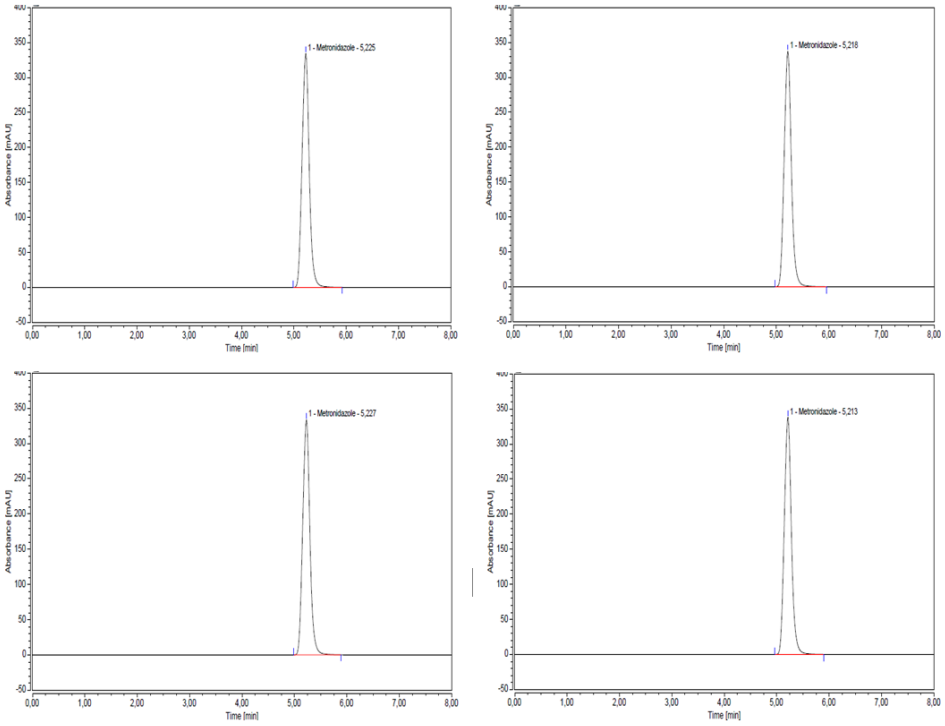

Figure 6: Chromatograms of M3 sample (4 injections)

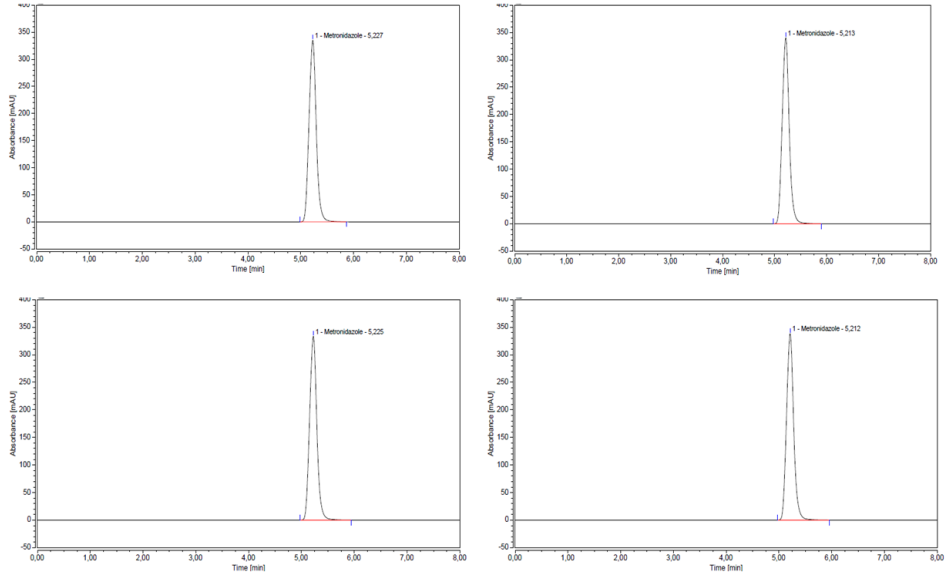

Figure 7: Chromatograms of M4 sample (4 injections)

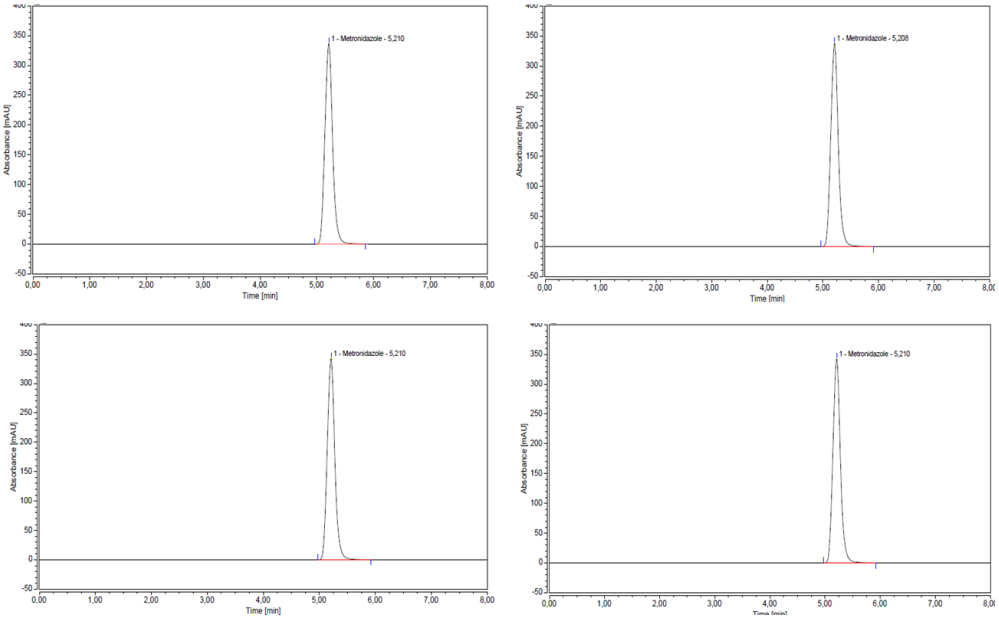

Figure 8: Chromatograms of M5 sample (4 injections)

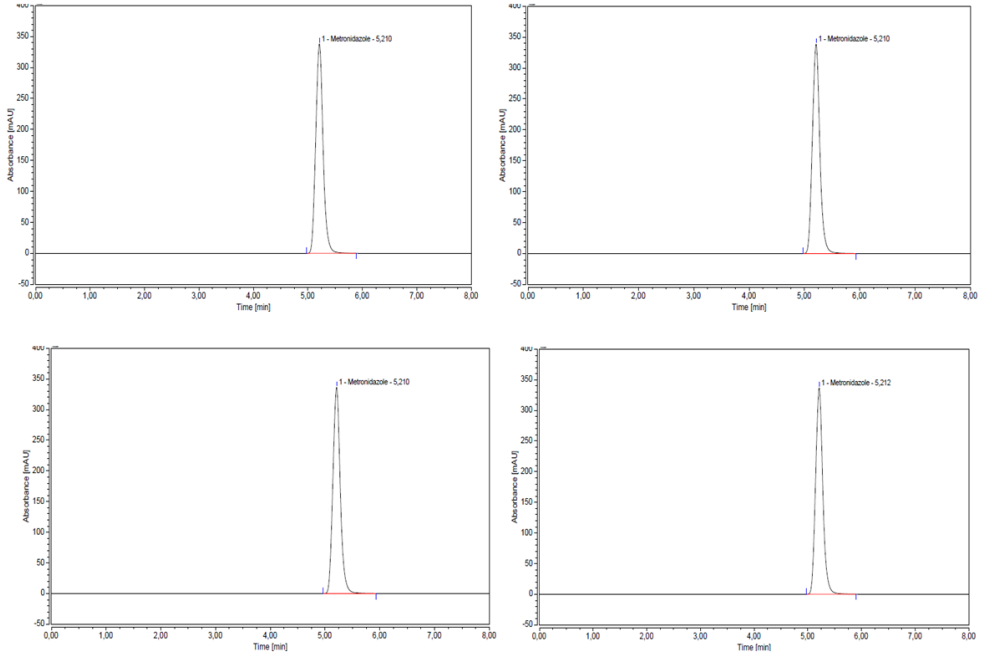

Figure 9: Chromatograms of M6 sample (4 injections)

and M6 samples. The **Table 2** summarizes the content results of *Metronidazole* API "As it is" and in "Anhydrous substance" by potentiometry of the various samples of *Metronidazole* analyzed as well as the standards required by the Eur Ph and USP [14]–[17].

The potentiometry determination of *Metronidazole* content shows that all the samples comply with the standards with the exception of M4 sample (98.92%) which had a slightly lower content but the confirmation by HPLC revealed that all samples had a content meets the required standards (**Table 1**)

Table 1: Content of *Metronidazole* "As it is" and in "Anhydrous substance" by HPLC.

Metronidazole sample	Weight	Pic Area (mAU.min)	"As it is" Content (%)	Loss on Drying (%)	"Anhydrous substance" content(%)	Average (%)	Norms in "Anhydrous substance" (%)
M1-P1-1	100,4	52,999	99,8	0,07	99,9		
M1-P1-2	100,4	53,252	100,3	0,07	100,4	**99,9**	
M1-P2-1	100,0	52,594	99,4	0,07	99,5		
M1-P2-2	100,0	52,832	99,9	0,07	100,0		
M2-P1-1	100,9	54,071	101,3	0,07	101,4		
M2-P1-2	100,9	54,209	101,6	0,07	101,6	**100,3**	99.0 à 101.0
M2-P2-1	100,2	52,471	99,0	0,07	99,1		
M2-P2-2	100,2	52,640	99,3	0,07	99,4		
M3-P1-1	100,3	53,006	99,9	0,50	100,4		
M3-P1-2	100,3	53,268	100,4	0,50	100,9	**100,0**	
M3-P2-1	100,5	52,921	99,6	0,50	100,1		
M3-P2-2	100,5	53,203	100,1	100,1	100,6		
M4-P1-1	100,1	53,327	100,7	0,81	101,5		
M4-P1-2	100,1	53,539	101,1	0,81	101,9	**100,6**	
M4-P2-1	100,1	52,949	100,0	0,81	100,8		
M4-P2-2	100,1	53,148	100,4	0,81	101,2		
M5-P1-1	100,5	52,922	99,6	0,38	99,9		
M5-P1-2	100,5	52,912	99,5	0,38	99,9	**100,4**	99.0 à 101.0
M5-P2-1	100,2	53,614	101,2	0,38	101,5		
M5-P2-2	100,2	53,679	101,3	0,38	101,7		
M6-P1-1	100,4	52,915	99,6	0,50	100,1		
M6-P1-2	100,4	52,881	99,6	0,50	100,1	**99,4**	
M6-P2-1	100,2	52,574	99,2	0,50	99,7		
M6-P2-2	100,2	52,521	99,1	0,50	99,6		

4. Conclusion

The content determination of six samples of *Metronidazole* API, collected from six pharmaceutical industries installed in Algeria, was realized by HPLC and potentiometric titration methods. The M1, M2, M3, M4 and M6 samples had a *Metronidazole* content meeting the standards required by the Eur Ph and USP. The evaluated methods showed to be adequate to quantify *Metronidazole* in the pharmaceutical raw material. The potentiometric titration did not

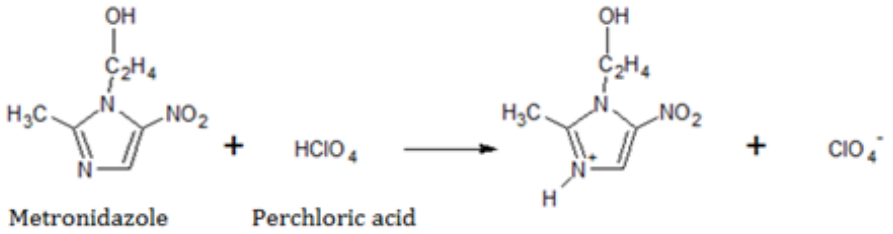

Figure 10: Reaction involved between *Metronidazole* and perchloric acid

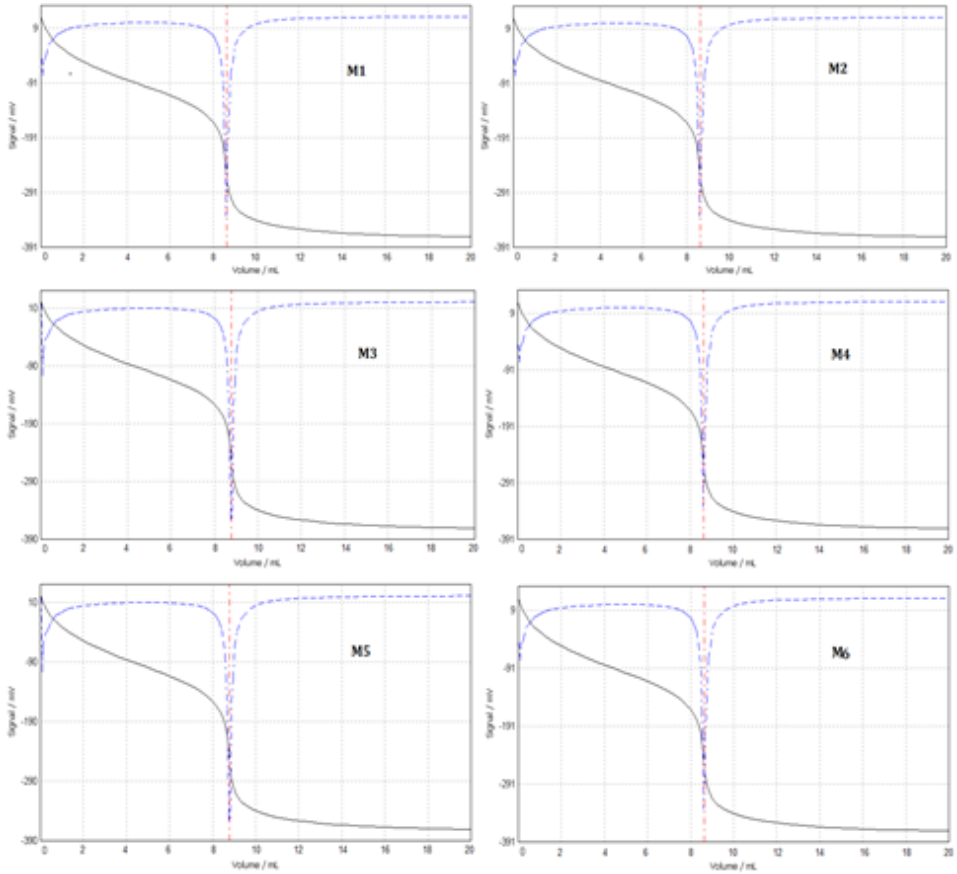

Figure 11: Graphics of potentiometric titration of M1, M2, M3, M4, M5 and M6 samples

required the reference substance use than the HPLC method, but the manipulation was rapid and the margin error was important than of HPLC method.

Table 2: Content of *Metronidazole* "As it is" and in "Anhydrous substance" by potentiometry

Metronidazole sample	Weight (mg)	HClO4 Volume (mL)	Correction Factor	Metronidazole Content (mg)	"As it is" Content (%)	Loss on Drying (%)	"Anhydrous Substance" Content (%)	Norms in "Anhydrous substance" (%)
M1	150,0	8,396	1,0465	150,42	100,28	0,07	100,35	99.0 à 101.0
M2	150,2	8,350	1,0465	149,60	99,60	0,07	99,67	
M3	150,7	8,453	1,0465	151,44	100,49	0,50	101,00	
M4	150,0	1,0465	147,18	98,12	0,81	98,92		
M5	150,4	8,415	1,0465	150,76	100,24	0,38	100,62	
M6	150,1	8,333	1,0465	149,29	99,46	0,50	99,96	

Disclosure of interest

The authors declare that they have no competing interests.

Authors' contribution

D. MATMOUR: Problematic, methodology, samples analysis, results interpretation, data statistics, writing original draft and corrections required. K.F.E. HASSAM: Samples analysis and results interpretation. N. HAMOUM, Y. MERAD and N. HAMDI ZIANI: Samples analysis. H. TOUMI: Samling and results interpretation.

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