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# Metoclopramide – nitrite reaction. Validation of its application to the spectrophotometric analysis of generic tablets

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***Abstract*—A quantitative method based on the product of the reaction between the antiemetic metoclopramide and sodium nitrite in acidic medium by visible spectrophotometry is proposed. Cooling or coupling stage are unnecessary, due to the stability of the colored diazo compound, which has a maximum absorbance at 380 nm. This procedure was applied to pharmaceutical formulations containing metoclopramide as single drug. Analytical validation of the proposed method compared to the reference method, HPLC – UV was performed. The parameters of specificity, precision (CV = 0.994% and 1.58% for repeatability and intermediate precision respectively), accuracy (recovery between 98.50 to 101.33%), linearity and linear range obtained, fall within the values established by the British Pharmacopoeia, USP and Argentinian pharmacopoeia.***

***Keywords*—Antiemetic, generic tablets, metoclopramide, spectrophotometric method, validation.***

## I. INTRODUCTION

As can be seen in its molecular structure (Fig 1) metoclopramide, 4-amino-5-chloro-N-[2 -(diethylamino) ethyl]-2-methoxybenzamide, (MCP), characterized as weak base [1-5] has a wide range of clinical applications in fields as diverse as gastroenterology, surgery, gynecology, radiology and cardiology. It exerts antiemetic effects, stimulating peristaltic emptying and gastrointestinal adjuvant. It is well absorbed when administered orally. It suffers very little transformation at the liver and plasmatic half-life is 2.6 to 6 hours in patients with normal renal function [6]. Metoclopramide hydrochloride is commonly used in prevention and relief of nausea and vomiting. Moreover, it is used in combination with chemotherapy, where drugs such as cisplatin, and other cytotoxic agents, are highly emetic [7].

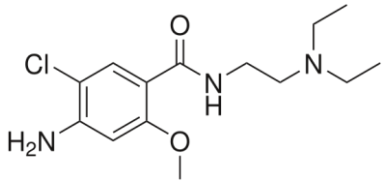


**Figure 1**: Molecular structure of metoclopramide

The British Pharmacopoeia [1], the U.S. Pharmacopoeia [2] and Argentinian Pharmacopoeia [3] establish high performance liquid chromatography (HPLC) with UV detection at 215 nm as quantitative assay method for the quality control of the tablets of MCP.

The great therapeutic importance of MCP in both clinical and experimental medicine has resulted in extensive literature on its determination in biological fluids and dosage forms, alone or in mixtures with other drugs; by HPLC [8], electron-capture gas chromatography [9], voltammetry [10-11], potentiometry [12] chemiluminescence spectrometry [13-16], fluorimetry[17], UV-spectrophotometry [18] or flow-injection spectrophotometry [19-20]. But some of the reported procedures are not simple for routine analysis and involve expensive experimental setup.

The determination of this drug by spectrophotometric methods has been proposed based on the formation of ion-pair complexes [21-22] and charge-transfer complexes [23-24].

A large set of methods is founded on diazotization of the aryl primary amine of MCP with $NaNO_2$ in acidic medium and the formation of a colored dye by the coupling reaction between various coupling agents: sodium dibenzoilmetane [25]; p-dimethylaminocinnamaldehyde [26]; imipramine hydrochloride [27] diphenylamine [28] acetyl acetone [29] N-(1-naphthyl)- ethylenediamine [30] p-nitroaniline [31-32]and α-naphthylamine [33]. The main difficulty is that solutions of nitrite are unstable and have to be stored in the refrigerator and frequently replaced, and it is necessary to attach the diazo compound to molecules that confer stability.

For routine quality control, a simple, rapid and sensitive spectrophotometric method is highly desirable. The proposed application uses nitrate solutions for *in situ* conversion into nitrite thus avoiding problems associated with nitrite oxidation. The nitrite generated is used as a chromogenic agent, obtaining a stable diazo compound at room temperature, which absorbs at 380 nm. Color stability is maintained for more than 2 hours, a period that exceeds the time required for their spectrophotometric reading.

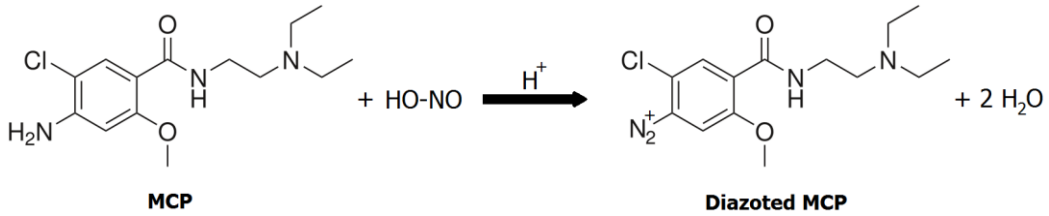


**Figure 2**: Diazotization of MCP whit HONO

Validation was performed. This process led to the quantification of MCP as major component in bulk and finished pharmaceuticals, therefore it is included in category I [1-3; 34]. Analytical attributes of accuracy, precision, specificity, linearity and linear range were determined.

The Pharmaceutical Plant of Corrientes (PLAMECOR), under the Ministry of Public Health of the province, produces MCP 10 mg tablets. These are distributed without cost in public hospitals and primary health centers.

The aim of the present work is to provide a simple, accurate, precise and inexpensive method for the assay of MCP performing the steps leading to its validation in bulk and pharmaceutical generic formulations compared to HPLC, in the presence of common excipients.

## II. EXPERIMENTAL

### 2.1Reagents and Samples

- Metoclopramide hydrochloride, active ingredient. Lot M102271. Origin: India. 99.8% purity
- Metoclopramide hydrochloride, 10 mg tablets, elaborated by PLAMECOR. Lot 039.
- Excipients: white precompressed powder (80%), fumed silica (1.00%), and talc (2.00%).
- Sodium nitrite, analytical reagent grade. Cicarelli (Argentina)
- Anhydrous sodium acetate, analytical reagent grade. Biopack (Argentina)
- Tetramethylammonium hydroxide, 25% solution. Merck (Germany)
- Hydrochloric acid 38%, analytical reagent grade. Cicarelli (Argentina)
- Glacial acetic acid, analytical reagent grade. Cicarelli (Argentina)
- Methanol, HPLC grade. Biopack (Argentina)
- Acetonitrile, HPLC grade. Biopack (Argentina)
- Phosphoric acid, analytical reagent grade. Cicarelli (Argentina)

### 2.2 Equipment

- UV-Visible Spectrophotometer Boeco S-26, range 190-900 nm.
- HPLC with variable UV wavelength detector Agilent 1120.

### 2.3 Spectrophotometric procedure

Stock solution of MCP was prepared dissolving an accurately weighted quantity of metoclopramide hydrochloride in distilled water to obtain a solution having a know concentration of 1 mg/mL. Working solutions of hydrochloric acid 6 M, and sodium nitrite 0.1 M were prepared. Standards contained aliquots of 1 to 5 mL of MCP stock solution; 1.0 mL of hydrochloric acid 6 M and 3.0 mL of sodium nitrite 0.1 M Blank solution was prepared using the same amount of reactive without MCP solution. Diazotization was carried out at room temperature and cooling was not necessary. These solutions were diluted to 50 mL with distilled water in a volumetric flask and homogenized. Absorption spectra of the diazo compound showed a maximum at 380 nm. Hence absorbance was recorded as function of concentration at 380 nm.

From a pool of 20 MCP tablets, an accurately weighted mass of 250.0 mg was dissolved by stirring for 15 minutes in 50 mL of distilled water. A 15 mL aliquot was centrifuged. Procedure described above was applied on supernatant.

The concentration of active ingredient in the sample was determined by calibration curve and referred to the average mass of one tablet (110.7 mg).

**2.4 Diazo compound stability**

The stability of the diazo compound was monitored by spectrophotometrical readings of a solution prepared as indicated in the previous item, considering time zero the mixture of reagents and active ingredient, recording readings every 10 min, for a period of 2h at room temperature.

**2.5 HPLC-UV procedure**

The method used an RP-18C column of 125 x 4.5 mm, flow rate of 1.5 mL/min and detection at 215 nm. Mobile phase was prepared by dissolving 2.7 g of sodium acetate in 500 mL of water, mixed with 500 mL of acetonitrile, and adding 2 mL of tetramethylammonium hydroxide solution in methanol (1:5). The solution was stirred, adjusted with glacial acetic acid to a pH of 6.5, filtered and sonicated for 10 min.

A stock solution of 1.0 mg/mL of active ingredient in 0.01 M phosphoric acid was prepared; afterwards an aliquot of this stock solution was quantitatively diluted with 0.01 M phosphoric acid to obtain a 50 μg/mL standard solution.

Sample solution preparation: 150 mg of pool of tablets were weighed, dissolved in 250 mL of phosphoric acid 0.01 M, and stirred for 10 min. An aliquot of 15 mL was centrifuged for 15 min. Supernatant and standard solution were injected into the HPLC (50 μL), recording the signal corresponding to the peak of MCP ($t_R$ = 2.98') at 215 nm. \

The concentration of MCP in solution of pool of tablets was calculated from the ratio of peak areas according to Equation 1:

$$C_{sample} = C_{std} \cdot A_{sample}/A_{std} \qquad \textbf{(1)}$$

**2.6 Spectrophotometric method validation**

**2.6.1 Specificity**

Specificity is the ability of a method to assess unequivocally the analyte in the presence of many others components, such as impurities, degradation products, matrix compound, etc. [1-3]. Specificity was assed by absorption spectra of reagents; reagents and placebo; and reagents and sample.

**2.6.2 Linearity**

Linearity represents the method's ability to produce results directly proportional to analyte concentration within a given interval. A calibration curve in 0.02 to 0.10 mg/mL interval was prepared using MCP standard solutions. Each analysis was performed by triplicate. This parameter was set using linear regression.

**2.6.3 Precision**

Precision is the degree of agreement among individual test results when the method is applied repeatedly to multiple aliquots of a homogeneous sample. It is expressed as coefficient of variation, CV, of a series of measurements. The precision shall be considered at two levels: repeatability and intermediate precision.

Repeatability expresses the precision under the same operating conditions in a short time interval. Twelve (12) determinations were conducted on the same sample, with a single operator, same equipment and in the same day and CV was determined.

Intermediate precision expresses the intra-laboratory variations. To evaluate this parameter a single homogeneous sample of one concentration was analyzed, and ratings were made by two analysts on two different days and taking three different aliquots from the sample solution. Global CV was determined

***2.6.4*** **Accuracy**

Accuracy is the proximity between the experimental results and the actual value. In this paper it was determined by two methods: recovery assay and comparison with the official HPLC- UV method [35].

## III. RESULTS AND DISCUSSIONS

**3.1 Stability**

After addition of the reagents, the diazo compound reaches maximum intensity immediately at room temperature. The color was stable for a period of more than 2 h (Fig. 3).

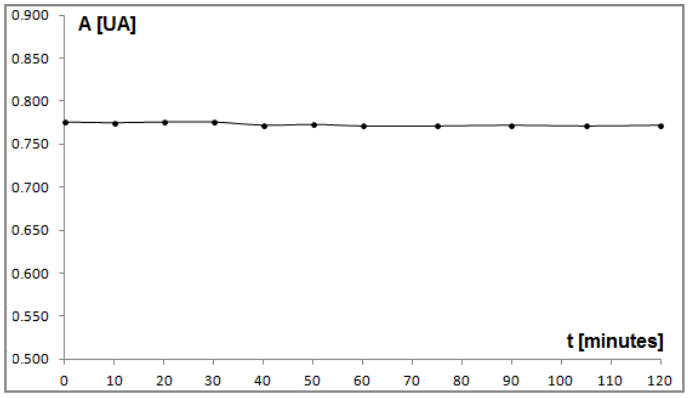


**Figure 3:** Stability of the MCP diazo compound

**3.2 Specificity**

The absorption spectra of reagents and reagent plus placebo solutions show many similarities between them (Fig. 4). This proves that the excipients that accompany the active ingredient in the formulation are chemically indifferent to the reagents used. The specificity of the method was confirmed.

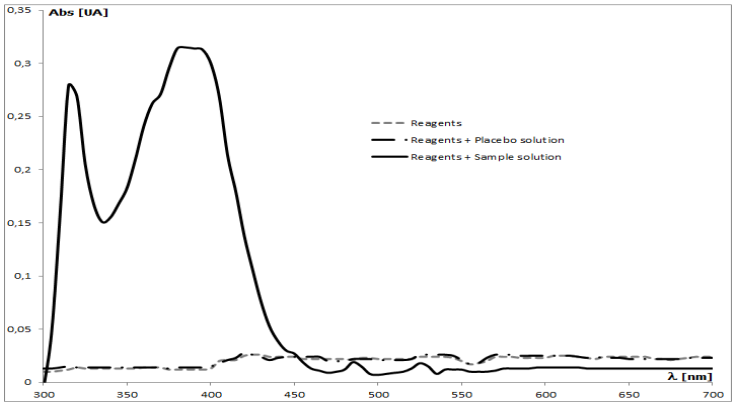


**Figure 4: Specificity assay.**

**3.3 Linearity**

The method was linear over the range of 0.02 to 0.10 mg/mL (Fig. 5). It was not necessary to explore the linearity of the response in a lower concentration range as it is quality control of the active ingredient of a drug.

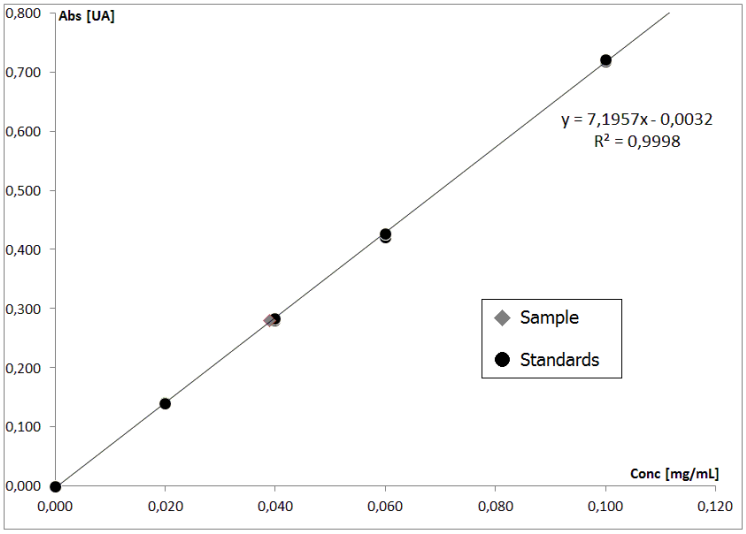

**Figure 5: S**pectrophotometric method linearity.

The confidence intervals for the intercept and slope were calculated using a Microsoft Excel ® spreadsheet, Table 1. The interval of the intercept includes the point (0, 0) with a confidence level of 95%.

**Table 1:** Analysis of variance of linear regression to a confidence level of 95% (p = 0.05)

| | |
|---|---|
| Adjusted Correlation $R^2$ | 0.9998 |
| Intercept | 0.0022 ± 0.0032 |
| Slope | 7.1957 ± 0.0568 |

### 3.4Repeatability.

According to the procedure described above, twelve sample aliquots were used. Results are shown in Table 2.

**Table 2: Repeatability of the proposed method**

| **N°** | **Mass recovered** [mg] |
|---|---|
| 1 | 9.81 |
| 2 | 10.02 |
| 3 | 10.02 |
| 4 | 9.81 |
| 5 | 9.88 |
| 6 | 9.74 |
| 7 | 9.70 |
| 8 | 9.91 |
| 9 | 9.81 |
| 10 | 9.88 |
| 11 | 9.77 |
| 12 | 9.84 |
| **X** | 9.85 |
| **SD** | 0.098 |
| **CV%** | 0.994 |

The method gives a CV% of 0.994; this is less than 2%, which is required for this type of analytical determinations [1-3].

**3.5 Intermediate Precision**

The study was carried out with spiked samples prepared with three different levels of MCP (5, 10 and 15 mg of active ingredient), following the spectrophotometric method as described. Each analysis was performed by triplicate. The results, expressed as recovered percentage (%Rec) of MCP, are shown in Table 3.

**Table 3:** Intermediate precision for the spectrophotometric method.

| Mass added [mg] | | ANALYST 1 | | ANALYST 2 | | REPRODUCIBILITY | |
|---|---|---|---|---|---|---|---|
| | | DAY 1 | DAY 2 | DAY 1 | DAY 2 | | |
| | | %Rec | %Rec | %Rec | %Rec | | |
| 5 | | 101.23 | 100.56 | 104.32 | 99.40 | | |
| | | 102.56 | 102.69 | 102.23 | 100.65 | **n =** | **12** |
| | | 100.89 | 101.20 | 102.23 | 98.89 | **M =** | **101.40** |
| | **M** | 101.56 | 101.48 | 102.93 | 99.65 | **SD =** | **1.50** |
| | **SD** | 0.88 | 1.09 | 1.21 | 0.91 | **CV% =** | **1.48** |
| | **RSD%** | 0.87 | 1.08 | 1.17 | 0.91 | | |
| 10 | | 97.46 | 101.22 | 100.95 | 103.65 | | |
| | | 99.67 | 102.95 | 99.65 | 102.66 | **n =** | **12** |
| | | 100.32 | 100.45 | 98.78 | 102.32 | **M =** | **100.84** |
| | **M** | 99.15 | 101.54 | 99.79 | 102.88 | **SD =** | **1.83** |
| | **SD** | 1.50 | 1.28 | 1.09 | 0.69 | **CV% =** | **1.82** |
| | **RSD%** | 1.51 | 1.26 | 1.09 | 0.67 | | |
| 15 | | 98.56 | 101.47 | 103.56 | 102.45 | | |
| | | 99.65 | 100.98 | 102.66 | 101.63 | **n =** | **12** |
| | | 101.56 | 99.89 | 100.37 | 99.12 | **M =** | **100.99** |
| | **M** | 99.92 | 100.78 | 102.20 | 101.07 | **SD =** | **1.52** |
| | **SD** | 1.52 | 0.81 | 1.64 | 1.74 | **CV% =** | **1.50** |
| | **RSD%** | 1.52 | 0.80 | 1.61 | 1.72 | | |

| **Global values (n = 36)** | |
|---|---|
| **M** | **101.08** |
| **SD** | **1.59** |
| **CV%** | **1.58** |

CV% for intermediate precision study was less than twice the RSD% of repeatability. Precision evaluated as repeatability and intermediate precision, shows satisfactory results [1-3].

**3.6 Accuracy**

**3.6.1 Recovery assay**

This assay was performed by triplicate and in three levels of concentration, Table 4. Placebo solutions enriched with different volumes of 1.0 mg/mL of MCP solution were analyzed.

**Table 4** – Results of the recovery assay

| Mass added [mg] | $M_{MCA}$ recovered [mg] | % Recovered | % Recovered average | Precision | |
|---|---|---|---|---|---|
| | | | | Parameter | Value [mg] |
| 1.00 | 0.984 | 98.36 | | **M** | 0.988 |
| 1.00 | 0.997 | 99.75 | 98.82 | **SD** | 0.008 |
| 1.00 | 0.984 | 98.36 | | **RSD%** | 0.811 |
| 2.00 | 2.024 | 101.21 | | **M** | 2.001 |
| 2.00 | 1.976 | 98.78 | 100.49 | **SD** | 0.024 |
| 2.00 | 2.003 | 100.17 | | **RSD%** | 1.217 |
| 3.00 | 2.981 | 99.38 | | **M** | 2.984 |
| 3.00 | 3.002 | 100.07 | 99.46 | **SD** | 0.017 |
| 3.00 | 2.967 | 98.92 | | **RSD%** | 0.585 |

The recovery was 98.50 to 101.33%, values that fall within the requirements set by USP and ANMAT (98.0-102.0%) [2-3].

**3.6.2 Comparison with the reference method**

Results obtained using the official method (HPLC) is shown in Table 5.

**Table 5:** Metoclopramide determination by HPLC-UV method.

| RUN | SIGNAL | CONC. [mg/mL] | Recovery [$mg_{CFX}$/tablet] | %Rec |
|---|---|---|---|---|
| Std | 3051.6 | 1.00 | - | |
| 1 | 3114.5 | 1.02 | 10.21 | 102.07 |
| 2 | 3074.9 | 1.01 | 10.08 | 100.77 |
| 3 | 3013.1 | 1.01 | 9.87 | 98.75 |
| 4 | 3082.1 | 1.01 | 10.10 | 101.01 |
| 5 | 3031.15 | 1.01 | 9.93 | 99.34 |
| 6 | 3068.5 | 1.01 | 10.06 | 100.56 |
| | | | **M** | 100.42 |
| | | | **SD** | 1.20 |
| | | | **RSD%** | 1.19 |

The spectrophotometric method was compared with the reference method using the F test for precisions and the t test for means, Table 6. Calculations were performed using Microsoft Excel ® spreadsheet.

**Table 6:** Statistical comparison between proposed and reference methods.
Values in parentheses correspond to those tabulated for p = 0.05.

| Parameter | Spectrophotometric Method | HPLC-UV Method |
|---|---|---|
| **X ± SD** | 9.925 ± 0.077 | 10.042 ± 0.123 |
| **RSD%** | 0.776 | 1.225 |
| **$S^2$** | 0.00591 | 0.01510 |
| **F test** | 2.554 (5.050) | |
| **t test** | 1.972 (2.228) | |

Statistically, it is found that there are no significant differences between the results obtained using the spectrophotometric method and the reference method for the determination of MCP, with a confidence level of 95%.

## IV. CONCLUSIONS

The results obtained enable the application of this spectrophotometric methodology for the quantification of the active ingredient in pharmaceutical tablets containing metoclopramide as a single drug, with accuracy and precision comparable to the HPLC reference method, without interference from common excipients and within the range established by the USP and ANMAT. The spectrophotometric method offers several advantages such as shorter time analysis, lower cost and simplicity.

## V. ACKNOWLEDGEMENTS

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