------ Raf. J. Sci., Vol. 25, No.2 pp. 10-21, 2014------

Development of Spectrophotometric Method for the Determination of Metoclopramide. HCl in a Pharmaceutical Preparations

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(Received 31 / 1 / 2013 ; Accepted 26 / 6 / 2013)

ABSTRACT

This paper includes a development of spectrophotometric method for the determination of metoclopramide.HCl. The method involves the diazotization of metoclopramide.HCl and coupling with pyridoxine. HCl, to form an intense orange colored, water-soluble and stable azo-dye which shows a maximum absorption at 470 nm. Beer's law is obeyed over the range 0.5-30 µg/ml metaclopramide. HCl with a molar absorptivity of $1.98*10^4$ $L.mol^{-1}.cm^{-1}$, The average recovery is 99.27% and relative standard deviation of ± 0.124 to ± 0.778%. This method has been applied successfully to the determination of metoclopramide.HCl in pharmaceutical preparations.

Keywords: Spectrophotometric method, metoclopramide.HCl, diazotization coupling method.

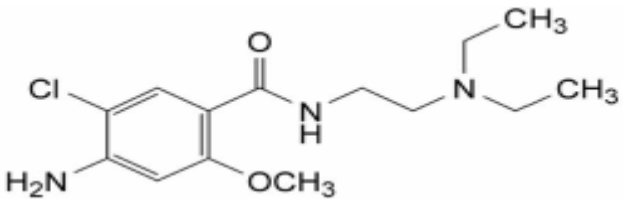
تطوير طريقة طيفية لتقدير الميتوكلوبراميد هيدروكلوريد في المستحضرات الصيدلانية

الملخص

يتضمن البحث تطوير طريقة طيفية لتقدير دواء الميتوكلوبراميد هيدروكلوريد، وتعتمد الطريقة على الاقتران الازوتي باستخدام كاشف البايردوكسين هايدروكلوريد لتكوين صبغة ازوية برتقالية اللون تقاس طيفيا عند 470 نانوميتر ذائبة في الماء ومستقرة، وكان المدى الخطي لقانون بير من 0.5 الى 30 مايكروغرام/مللتر وبقيمة معامل امتصاص مولاري 1.98×10^4 لتر.مول$^{-1}$.سم$^{-1}$ ومعدل نسبة استرجاع 99.27 وقيمة انحراف قياسي نسبي يتراوح بين 0.124 و0.788، وطبقت الطريقة بنجاح على المستحضرات الصيدلانية للميتوكلوبراميد.

INTRODUCTION

Metoclopramide.HCl is 4-amino-5-chloro-2-methoxy-N-(2-diethyl-aminoethyl) benzamide (British Pharmacopeia, 2008).



.HCl.H_2O

M.Wt=354.3 g/ mol

$C_{14}H_{22}ClN_3O_2.HCl.H_2O$

Metoclopramide.HCl is an antiemetic and gastroprokinetic agent. It is commonly used to treat nausea and vomiting, to facilitate gastric emptying in people with gastroparesis, and as a treatment for the gastric stasis often associated with migraine headaches. Metoclopramide is commonly used to treat nausea which is due to chemotherapy and that occurring post operatively. Evidence also supports its use for gastroparesis (poor stomach emptying) and gastroesophageal reflux disease. It is used to treat nausea and vomiting associated with conditions such as uremia, radiation sickness, malignancy, labor, infection, migraine headaches, and emetogenic drugs (Marietta, 2009; Roth, 2007). It is available under various trade names including Maxolon, Reglan, Degan, Maxeran, Primperan, Pylomid, Plazilin, Cerucal, Pramin, Plasil and Pulin (Wikipedia, 2012).

The spectrophotometric methods are the most analytical methods used for metoclopramide determination using different reagents including: promethazine in presence of hypochlorite (Ahmad and Ali, 2006), flouranil (Al-Ghabsha *et al*., 2004), 4-dimethylaminobenzaldehyde (Patel *et al*., 2006), 1,10-phenathraline or bipyridyl in the presence of Fe(III) or Ce(IV) ions (Amin and Ragab, 2003), 2,4-dinitroflourobenzene (Al-Hamody and Al-Sabha, 2006), 4-dimethylaminecinnamaldehyde (Moussa, 2000), pyrocatecol (Nabeel *et al*., 2011), 8-hydroxyquinoline (Al-Abbassi *et al*., 2011) .

Other reported methods include titrimetry (British Pharmacopeia, 2008), (Vasiliev, 1968), flameless atomic absorption spectrophotometry (Park *et al*., 1980). In addition, there are other methods used for the determination of metoclopramide.HCl such as: GC-MS (Riggs *et al*., 1994), HPLC (Nieder and Jaeger, 1987), (Lee *et al*., 1990), reverse phase - high performance liquid chromatogramphy (Shields and Mackichan, 1990), and flow injection method (Fan *et al*., 2005) .

The present method involves a spectrophotometric method for the determination of metoclopramide.HCl by diazodization coupling with pyridoxine.HCl. A soluble and stable colored dye was formed which can be measured at 470 nm. The method does not require a temperature control or a solvent extraction step and can be applied successfully to pharmaceutical preparations containing metoclopramide.HCl.

EXPERIMENT

Apparatus

All spectral and absorbance measurements were performed on Shimadzu UV-Visible 160 double beam recording spectrophotometer using 1-cm silica cells. pH meter type Philips PW 9420 was used for pH reading.

Reagents

All chemicals used in this study were of analytical reagent grade, and MCP metaelopramide material was provided from general establishment for medical appliance and Drugs / SDI – Samaraa / Iraq.

Standard Solutions

Metaclopramide solution 100 ppm. This solution is prepared by dissolving 0.01 g of metoclopramide in100 mL of distilled water in a volumetric flask. The solution was kept in a brown bottle.

Hydrochloric acid solution, ~1N. This solution is prepared by diluting 8.3 mL of concentrated hydrochloric acid with distilled water in a100 mL volumetric flask.

Sodium nitrite solution, 1%. This solution was prepared by dissolving 1 g of sodium nitrite (BDH) using distilled water in a100-mL volumetric flask. The solution was kept in a brown bottle and it was stable for at least one week.

Sulphamic acid solution, 3. This solution was prepared by dissolving 3 g of sulphamic acid (fluka) using distilled water in a100-mL volumetric flask. The solution was kept in a brown bottle, and it was stable for at lest one week.

Sodium carbonate, 1N. This solution was prepared by dissolving 5.5 g of sodium carbonate in distilled water then completing the volume to 100 mL in volumetric flask.

Pyridoxine hydrochloride solution 1%. This solution was prepared by dissolving 1 g of pyridoxine hydrochloride in distilled water then completing the volume to 100-mL in a volumetric flask. The solution was kept in a brown bottle and it was stable for at least one week.

Foreign compound solutions, 1000 µg/ml. These solutions are prepared by dissolving 0.1g of the compound in distilled water and the volume was completed to 100-mL in a volumetric flask.

Procedure and calibration graph. To a series of 20 mL volumetric flask, 0.1-6 mL of 100 µg/mL metoclopramide hydrochloride solution were transferred, followed by 1 mL of 1N hydrochloric acid and 1 mL of 1% sodium nitrite solution, the mixture was allowed to stand for 3 minutes and then 1 mL of 3% sulphamic acid solution was added with occasional shaking for another 3 minutes, after that a 2.5 mL of 1% pyridoxine hydrochloride solution and 2 ml of sodium carbonate were added. After the volumetric flasks were completed to the mark with distilled water, the absorbance was measured at 470 nm against the reagent

blank solution after 15 minutes. A linear calibration graph was obtained over the concentration range of 0.5-30 µg/mL metoclopramide hydrochloride (Fig.1). The molar absorptivity has been found to be $1.98*10^4$ $L.mol^{-1}.cm^{-1}$.

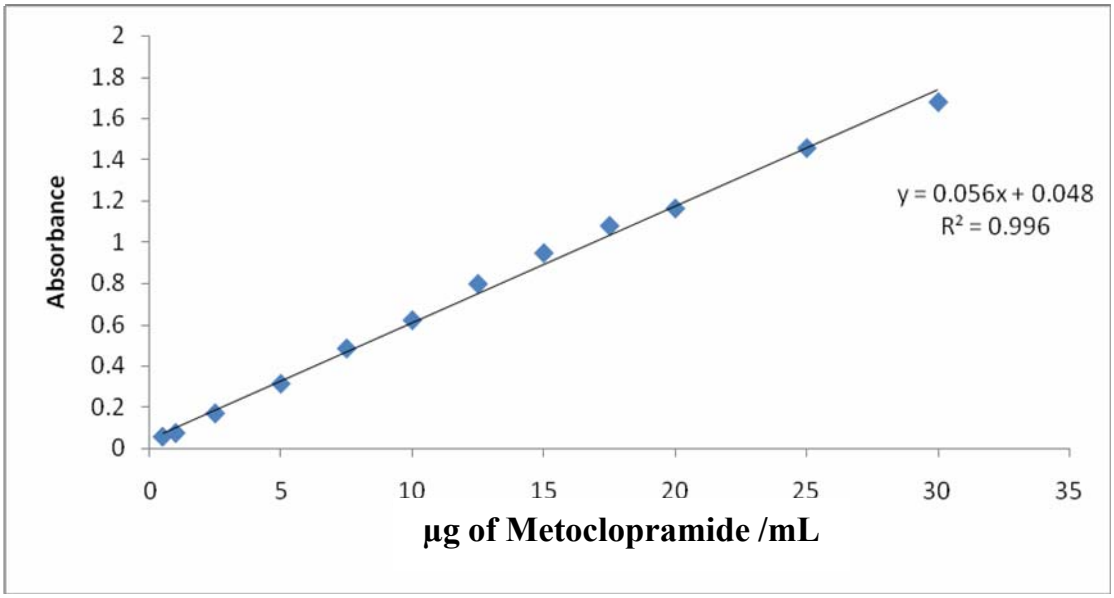


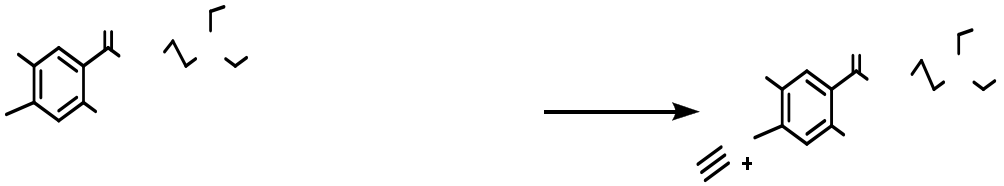
Fig. 1: Calibration graph of metoclopramide hydrochloride determination

RESULTS AND DISCUSSION

For the subsequent experiments, 10 µg/mL metoclopramide hydrochloride was taken .

Principle of the method

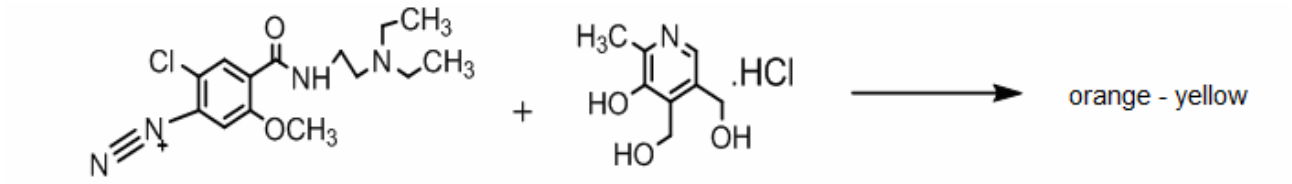
Metoclopramide hydrochloride, in acidic medium, was allowed to react with excess nitrite to form the corresponding diazonium salt:



After the removal of the residual nitrite with sulphamic acid:

$$HNO_2 + H_2N - SO_3H \rightarrow N_2 \uparrow + H_2O + H_2SO_4$$

The diazotized metoclopramide hydrochloride was then coupled with pyridoxine to form, an intensely orange-yellow colored dye:



Diazotised metoclopramide **Pyridoxine**

Effect of acids

The effect of different amounts of different acids has been investigated to examine their effect on the intensity of the colored azo dye. The results are shown in Table (1).

Table 1: Effect of diazotization acid on the absorbance

Acid used (1 N)	Absorbance /mL of acid added				
	0.5	**1**	**1.5**	**2**	**2.5**
HCl	0.375	0.380	0.376	0.371	0.370
HNO_3	0.305	0.308	0.310	0.311	0.306
H_2SO_4	0.366	0.369	0.372	0.365	0.360
H_3PO_4	0.370	0.375	0.366	0.361	0.352
CH_3COOH	0.355	0.359	0.345	0.341	0.335

The results show, that 1 mL of 1 N hydrochloric acid solution gives the best result.

Effect of sodium nitrite amount and time

The maximum absorbance reading was obtained by adding 0.2 mL of 1% sodium nitrite with 5 minutes reaction time.

Effect of sulphamic acid amount and time

The excess of nitrous acid is removed by the addition of sulphamic acid solution. The sulphamic acid amount and time allowed have been studied and the results show that the maximum absorbance reading is obtained by adding 0.1 mL of 3% sulphamic acid after 2 minutes reaction time.

Effect of pyridoxine.HCl amount

The effect of different amounts of 1% pyridoxine.HCl solution has been studied on the intensity of the formed azo dye; 2.5 mL of 1% of pyridoxine.HCl was used for the subsequent experiments since it gave maximum absorbance.

Effect of base amount

This investigation showed that the azo dye is formed in alkaline medium, therefore a different types and amounts of strong and weak bases have been studied [Table (2)]. The results indicate that a volume of 2 mL of 1 N sodium in the procedum, it is sodium carbonate hydroxide gives maximum absorbance.

Table 2: Effect of base amount

Base solution used 1N	Variable		Absorbance / mL of base used				
		0.5	1	1.5	2	2.5	3
NaOH	A	0.191	0.454	0.585	0.501	0.411	0.392
	$\Delta\lambda^*_{(nm)}$	78	164	150	152	109	111
	pH	1.89	4.95	11.94	12.12	12.28	12.42
KOH	A	0.160	0.199	0.557	0.527	0.520	0.493
	$\Delta\lambda_{(nm)}$	79	76	149	154	154	154
	pH	1.81	2.26	11.39	12.17	12.37	12.51
Na_2CO_3	A	0.150	0.219	0.586	0.615	0.590	0.522
	$\Delta\lambda_{(nm)}$	79	77	173	166	162	162
	pH	1.76	2.15	5.70	6.25	6.66	6.75
$NaHCO_3$	A	0.123	0.104	0.146	0.460	0.516	0.536
	$\Delta\lambda_{(nm)}$	12	84	84	94	101	100
	pH	1.82	1.87	2.08	4.92	7.64	8.10

*$\Delta\lambda = \lambda_{max\ of\ sample} - \lambda_{max\ of\ blank}$

Effect of time:

The colored azo dye was developed immediately after the addition of the base and exhibits maximum intensity at room temperature. The colour was stable for at least 60 minutes and the results are given in Table (3).

Table 3: Effect of time allowed

Time (min.)	Absorbance
5	0.610
10	0.612
15	0.612
20	0.614
25	0.614
30	0.615
35	0.615
40	0.615
45	0.615
50	0.615
55	0.616
60	0.617
65	0.619
70	0.619

Final absorption spectra:

Under the above established optimized conditions, absorption spectra of the azo dye formed in the reaction mixture against its corresponding reagent blank and of the blank

against distilled water are recorded and shown in (Fig. 2). The colored dye exhibits an absorption maxima at 470 nm against reagent blank.

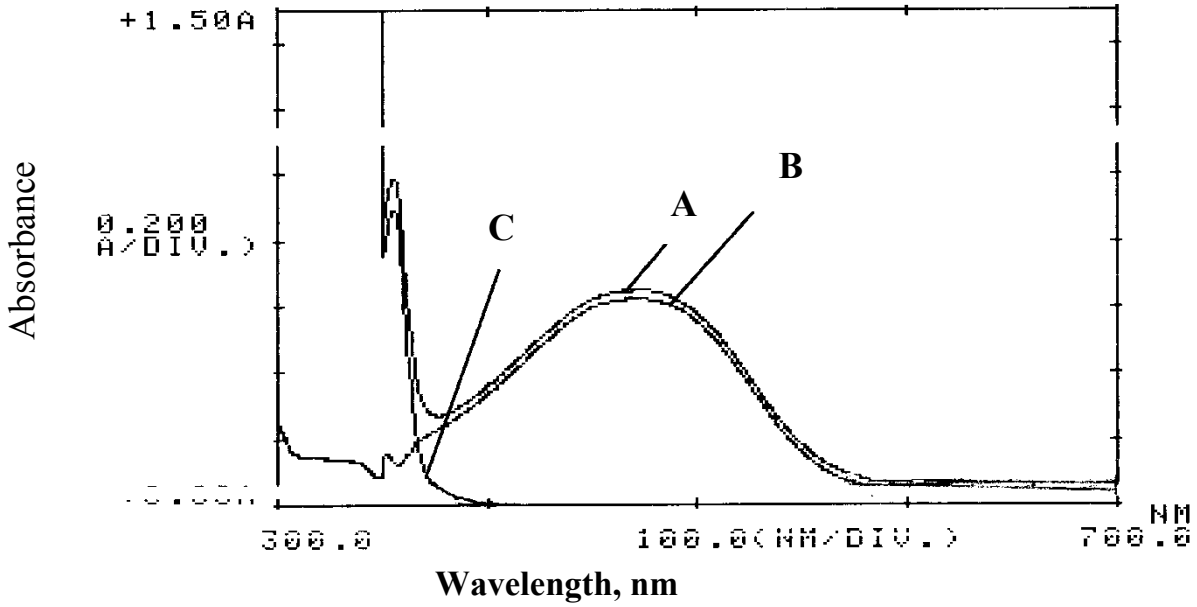


Fig. 2: Absorption spectra of 10 µg of metoclopramide hydrochloride treated according to the recommended procedure and measured against
(A) sample against distilled water
(B) sample against blank
(C) blank against distilled water

Accuracy and precision

To check the accuracy and precision of the calibration graph; metoclopramide hydrochloride was determined at three different concentrations and the results are shown in Table (4), which indicate a good accuracy and precision.

Table 4: Accuracy and precision

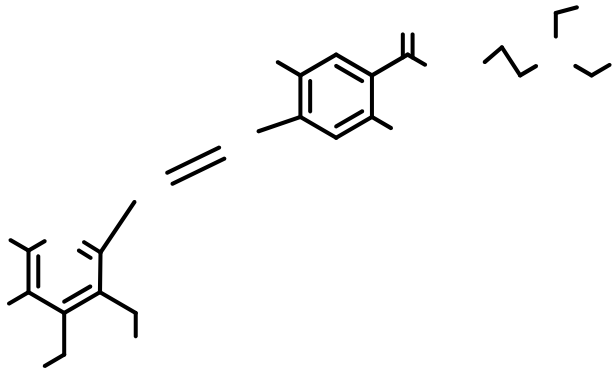
Amount of metoclopromide.HCl (µg/mL)		Recovery* (%)	RSD* (%)
added	found		
5	4.80	96.0	±0.778
10	10.25	102.5	±0.124
20	19.86	99.3	±0.179

*Average of four determinations

Nature of the azo dye:

The composition of the intense orange azo-dye has been established using job s' method of continuous variations and the mole-ratio method.

The results indicate that the azo-dye has been formed in the ratio of 1:1 (metoclopromide.HCl-pyridoxine.HCl), and the azo dye may have the following suggested structure:



Orange azo dye-

The average stability constant of the dye in aqueous solution under the established experimental condition, has been calculated and found to be 45.35×10^{6} L/ mol .

Effect of organic solvents:

The spectrophotometric characteristics of the azo dye in different organic solvents are given in Table (5).

Table 5: Spectrophotometric characteristics of the azo dye in various organic solvents

Solvent	λ_{max} , nm	Absorbance
Acetic acid	489	0.416
Acetone	Turbid	Turbid
DMF	Turbid	Turbid
Ethanol	474	0.581
Methanol	Turbid	Turbid
Water	470	0.611

Water is shown to be a good medium from the point view of sensitivity and economy, therefore it has still been recommended for dilution.

Study of interferences:

In order to realize the analytical application of this method, effects of foreign compounds have been studied by carrying out the determination of metoclopramide.HCl in the presence of each interferant using the recommended procedure. The obtained results are shown in Table (6).

Table 6: Effect of interferences on the determination of metoclopramide.HCl

Interferences	Recovery % of 200 µg metoclopramid per µg interferent added		
	100	**500**	**1000**
Glucose	100.3	100.4	100.9
Lactose	100.1	100.4	100.8
Starch	100.0	100.1	100.8
Acacia	100.9	100.4	100.0

The experimental results showed that there were no interference from excipients for the examined method.

Application of the method:

To test the applicability of the present method, it has been applied to the determination of metoclopramide.HCl in pharmaceutical preparations, the results are shown in Table (7).

Table 7: Determination of metoclopramide.HCl in pharmaceutical preparations

Drug	Pharmaceutical preparation	Amount present (µg/mL)	Amount measured (µg/mL)	Recovery* (%)	Drug found
Metoclopramid hydrochloride	Metoclopramide-HCl/ (5mg/ tablet), SDI- Iraq	5	4.82	96.4	4.820(mg)
		10	10.14	101.4	5.070(mg)
		20	19.98	99.9	4.995(mg)
	Metoclopramide-HCl Injection (10mg /2mL, Ibn-Hayan-Syria	5	4.88	97.6	9.760(mg/2mL)
		10	10.25	102.5	10.250(mg/2mL)
		20	19.88	99.4	9.940(mg/2mL)
	Metoclopramide-HCl/ Syrup (5mg/5mL) Arab pharmaceutical anufacturing Co. Ltd., sult-Jordan	5	4.75	95.0	4.750(mg/5mL)
		10	10.29	102.9	5.145(mg/5mL)
		20	19.28	96.4	4.820(mg/5mL)

*recovery of three determinations

The results in Table (7) indicate a good applicability of the method.

Comparison of the methods

Table (8) shows the comparison of analytical variables obtained for the present method with those of the recent spectrophotometric methods.

Table 8: Comparison of the methods

Analytical parameters	Present method	Literature Method (Ahmad, 2010)	Literature Method (Mahmood *et al*., 2007)
pH	6.66	10.03	Alkaline
Temperature (°C)	Room temperature	Room temperature	Room temperature
λ_{max} (nm)	470	450	549
Medium of reaction	Aqueous	Aqueous	Aqueous
Reagent	Pyridoxine	2,4- Dihydroxy-acetophenone	α-Naphthol
Beer's law range (ppm)	0.5-30	0.4-12	0.5-8
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.98×10^4	2.48×10^4	3.85×10^4
RSD (%)	≤±0.778	≤±1.092	≤±2.17
Nature of the dye	1:1	1:1	1:1
Stability of the colour (minutes)	70	60	60
Colour of the product	Orange	Orange	Violet
K ($Molar^{-1}$)	4.535×10^7	2.4×10^5	9×10^4
Nature of the dye	1:1	1:1	1:1
Application of the method	Has been applied to the assay of metoclopramide hydrochloride in pharmaceutical preparations (tablets, injection and syrup)	Has been applied to the assay of metoclopramide hydrochloride in pharmaceutical preparations (tablets, injection and syrup)	Has been applied to the assay of metoclopramide hydrochloride in pharmaceutical preparations (syrup, mouth drop, tablet and injection)

CONCLUSION

A sensitive and simple spectrophotomitric method for the determination of metoclopramide.HCl drug in aqueous solution has been investigated; it is based diazotization of metoclopramide.HCl and couping with pyridoxine.HCl to form an intense orange coloured, water-soluble and stable azo dye which exhibits a maximum absorption at 470 nm. The proposed method requires neither temperature control ,nor solvent extraction and it can be applied successfully to the determination of the drug in pharmaceutical preparation

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