A SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF MOSAPRIDE BY DIAZOTIZATION METHOD

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ABSTRACT. A facile spectrophotometric method for the determination of mosapride is described. This method is based on the reaction of mosapride with nitrite in acid medium to form diazonium ion, which is coupled with acetylacetone or ethyl acetoacetate in basic medium to form azo dyes, which shows absorption maxima at 402 or 422 nm. The method obeys Beer's law in the concentration range of 0.4-13.5 μgml^{-1} of mosapride with acetylacetone and 0.5-17.0 μgml^{-1} of mosapride with ethyl acetoacetate. The molar absorptivity and Sandell's sensitivity of mosapride-acetylacetone and mosapride-ethyl acetoacetate azo dyes are $1.026 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$, $4.484 \times 10^{-3} \mu\text{gcm}^{-2}$ and $1.097 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$, $4.193 \times 10^{-3} \mu\text{gcm}^{-2}$ respectively. The dye formed is stable for 8 hrs. The optimum reaction conditions and other analytical parameters are evaluated. No interference observed from common excipients. The method has been successfully applied to the determination of mosapride in pharmaceutical samples.

KEYWORDS: *spectrophotometry, diazotization, mosapride, nitrite, acetylacetone, ethyl acetoacetate.*

1. INTRODUCTION

Gastroprokinetic agents have an important role to play in conjunction with life style modifications in the short and long term medical management of gastroesophageal reflux disease (GERD) [1]. The prevalence of GERD and dyspepsia is increasing in many Asian countries [2]. Mosapride citrate, chemically 4-amino-5-chloro-2-ethoxy-N-[[4-[(4-fluorophenyl) methyl]-2-morpholinyl]-methyl benzamide is a potent

gastroprokinetic drug. It behaves as a selective 5-HT₄- receptor agonist [3] and enhances only upper gastroprokinetic motor activity [4,5].

Few methods have been reported for the determination of mosapride such as spectrofluorometry [6] and liquid chromatography [7-9]. Spectrophotometric method for the determination of mosapride

by diazo and coupling reaction is reported earlier [10-13]. The reagents reported for the spectrophotometric determination of mosapride are less selective [13], less sensitive [10] and some require stringent experimental conditions and are carcinogenic in nature [10]. In the present work a facile, selective method for the determination of mosapride in pharmaceutical samples by spectrophotometric method is described. The amino group in mosapride is diazotized with nitrite in acidic medium at room temperature and the diazonium salt thus formed is coupled with acetylacetone or ethyl acetoacetate to give water-soluble colored dye in alkaline conditions is the basis for the determination of mosapride. The method has been successfully applied to the determination of mosapride in pharmaceutical samples.

2. EXPERIMENTAL

A SHIMADZU Deutschland GmbH UV-2550 spectrophotometer with 1cm quartz cell was used for the absorbance measurements and a WTW pH 330, pH meter were used. All chemicals used were of analytical reagent grade (Merck) or chemically pure grade and double distilled water was used for the dilution of reagents and samples. A standard solution of mosapride (Gift sample from Cipla Ltd, Mumbai, India) was prepared by dissolving the required amount of the sample in absolute alcohol and diluted with distilled water. Working solution was prepared as required by dilution. Sodium nitrite solution (0.05%), acetylacetone (2%), ethyl acetoacetate (2%), hydrochloric acid (0.2 M), sodium hydroxide (2 M), EDTA (0.2 M) and sodium carbonate (1%) were used.

2.1 GENERAL PROCEDURE FOR THE DETERMINATION OF MOSAPRIDE

An aliquot of the sample solution containing known amount of mosapride was transferred

into a series of 10 ml calibrated flasks. To this 1 ml of 0.05 % solution of sodium nitrite and 0.5 ml of 0.2 M hydrochloric acid were added and the solution was shaken thoroughly for 2 minutes and kept aside for completion of diazotization reaction. Then, volumes of 1ml of 2% acetylacetone or ethyl acetoacetate and 2 ml of 2 M sodium hydroxide solutions were added and the contents were diluted to 10 ml using double distilled water and mixed well. After 5 minutes, the absorbance of the colored azo dye formed were measured at 402 or 422 nm against the reagent blank.

3. RESULTS AND DISCUSSION

The method involves the diazotization of mosapride with nitrite, followed by the coupling of acetylacetone or ethyl acetoacetate in alkaline medium. The reaction system is represented in Fig. 1.

3.1 EFFECT OF ACID CONCENTRATION AND TEMPERATURE ON DIAZOTIZATION

Diazotization was carried out at room temperature ($25 \pm 5^\circ\text{C}$). The effect of acidity on the diazotization reaction was studied in the range 0.1- 0.5 M hydrochloric acid, and constant absorbance was observed in this range. Above this range, a decrease in the absorbance was observed. The optimum acidity for the diazotization was fixed to be 0.2 M and minimum time for the complete diazotization was found to be 2 minutes.

3.2 EFFECT OF THE NITRITE CONCENTRATION

The effect of the nitrite concentration on the color intensity was studied using the proposed procedure and adding 1 ml of 0.01-0.1 % solutions of the nitrite in hydrochloric acid (0.2 M) to a series of nitrite solutions. The results showed that a 1 ml of 0.05% of nitrite solution was sufficient for complete color development. Higher concentration did not

enhance the absorbance further, and lower concentration did not give good results.

3.3 EFFECT OF COUPLING AGENT

The effect of varying the concentration of coupling agent was studied using the proposed procedure and adding 0.2-2.0 ml of 2% acetylacetone or 0.2-2.0 ml of 2% ethyl acetoacetate to a series of nitrite solutions. It was found that a stable color was formed by adding 1ml of either acetyl acetone (2%) or ethyl acetoacetate (2%) solution in a final volume of 10 ml.

3.4 EFFECT OF SODIUM HYDROXIDE CONCENTRATION

The effect of sodium hydroxide concentration on the absorbance was studied; The effects of adding different volumes of 0.5-2.0 ml of 2 M sodium hydroxide solutions were examined. The investigations showed that 1.0-1.2 ml of NaOH gave maximum absorbance and 1.0 ml of 2 M NaOH solutions was chosen for the procedure. Other alkaline solutions were tried, but best results were obtained by using sodium hydroxide.

3.5 ANALYTICAL DATA

The adherence to Beer's law was studied by measuring the absorbance values of solutions of variable mosapride concentrations. A straight line graph was obtained by plotting absorbance against concentration of mosapride. This method obeys Beer's law in the concentration range of 0.4-13.5 μgml^{-1} for mosapride with acetylacetone and 0.5-17.0 μgml^{-1} for nitrite with ethyl acetoacetate couples. The molar absorptivity, sandell's sensitivity of colored system with nitrite-acetylacetone and nitrite-ethyl acetoacetate couples were found to be $1.026 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$, $4.484 \times 10^{-3} \mu\text{gcm}^{-2}$ and $1.097 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$, $4.193 \times 10^{-3} \mu\text{gcm}^{-2}$

respectively. The detection limit ($D_L = 3.3\sigma/S$), quantitation limit ($Q_L = 10\sigma/S$) [where σ is the standard deviation of the reagent blank ($n=5$) and S is the slope of the calibration curve] and correlation coefficient for the nitrite determination with nitrite-acetylacetone diazocouple and nitrite-ethyl acetoacetate diazocouple were found to be 0.137 μgml^{-1} , 0.417 μgml^{-1} and 0.9996 and 0.141 μgml^{-1} , 0.427 μgml^{-1} and 0.9994 respectively.

4. APPLICATIONS

The proposed method was applied to the determination of mosapride in pharmaceuticals samples. The results of assay compare favorably with the reference method. (Table. 1 & 2). Statistical analysis of the results by t and F-tests showed no significant difference in accuracy and precision of the proposed and reference method [14]. The other active ingredients and excipients usually present in pharmaceutical sample forms did not interfere. The reliability of the method to analyze pharmaceutical samples was checked by recovery experiments, which gave quantitative results with good reproducibility.

5. CONCLUSIONS

The proposed method for the determination of mosapride is simple, sensitive and reliable has a wide analytical range without the need for extraction or heating. The reagent proposed has the advantage of high sensitivity and low absorbance of reagent blank. The developed method does not involve any stringent reaction conditions and offers the advantages of high color stability (8 hrs). The proposed method has been successfully applied to the determination of mosapride in various pharmaceutical samples.

Fig.1. Presentation of the reaction scheme

TABLE. 1: DETERMINATION OF MOSAPRIDE IN DIFFERENT PHARMACEUTICAL SAMPLES USING DIAZOCOUPLE OF NITRITE WITH ACETYLACETONE

Samples	SMP taken ($\mu\text{g ml}^{-1}$)	Proposed method		Reference method		t- test ^b	F-test ^c
		SMP found $\pm \text{SD}^a$ ($\mu\text{g ml}^{-1}$)	Recovery %	SMP found $\pm \text{SD}^a$ ($\mu\text{g ml}^{-1}$)	Recovery %		
Somapride (5 mg/tab)	4.0	4.01 ± 0.04	100.25	3.96 ± 0.05	99.0	1.234	1.56
	8.0	7.98 ± 0.03	99.75	7.95 ± 0.02	99.38	0.832	2.25
	12.0	11.98 ± 0.04	99.83	11.93 ± 0.05	99.42	1.231	1.56
Mozax (5 mg/tab)	4.0	3.95 ± 0.08	98.75	3.97 ± 0.06	99.25	0.376	1.77
	8.0	7.99 ± 0.03	99.88	8.01 ± 0.05	100.13	0.542	2.77
	12.0	11.99 ± 0.06	99.92	11.97 ± 0.05	99.75	0.404	1.44

Mosid (5 mg/tab)	4.0	3.97 ± 0.04	99.25	3.95 ± 0.07	98.75	0.392	3.06
	8.0	7.99 ± 0.06	99.87	7.96 ± 0.05	99.50	0.607	1.44
	12.0	12.00 ± 0.04	100.08	11.98 ± 0.02	99.83	0.707	4.00
Moza (5 mg/tab)	4.0	4.02 ± 0.06	100.05	4.00 ± 0.08	100.00	0.316	1.77
	8.0	7.95 ± 0.04	99.37	7.98 ± 0.05	99.75	0.741	1.56
	12.0	11.94 ± 0.08	99.50	11.92 ± 0.10	99.33	0.246	1.56
Mozasef (5 mg/tab)	4.0	4.03 ± 0.03	100.75	3.99 ± 0.04	99.75	1.264	1.77
	8.0	7.98 ± 0.06	99.75	7.97 ± 0.05	99.62	0.202	1.44
	12.0	11.93 ± 0.05	99.42	11.91 ± 0.04	99.25	0.490	1.56

a. Mean (SMP-Samples) ± standard deviation(n=5)

b. Tabulated t-value for 8 degree of freedom at P(0.95) is 2.306.

c. Tabulated F-value for (4,4) degree of freedom at P(0.95) is 6.39.

TABLE. 2: DETERMINATION OF MOSAPRIDE IN DIFFERENT PHARMACEUTICAL SAMPLES USING DIAZOCOUPLE OF NITRITE WITH ETHYL ACETOACETATE

Samples	SMP added ($\mu\text{g ml}^{-1}$)	Proposed method		Reference method		t- test ^b	F-test ^c
		SMP found ± SD ^a ($\mu\text{g ml}^{-1}$)	Recovery %	SMP found ± SD ^a ($\mu\text{g ml}^{-1}$)	Recovery %		
Somapride (5 mg/tab)	5.0	4.96 ± 0.01	99.20	4.94 ± 0.02	98.80	1.414	4.00
	10.0	9.99 ± 0.02	99.90	9.98 ± 0.04	99.80	0.353	4.00
	15.0	14.95 ± 0.06	99.67	14.96 ± 0.05	99.73	0.202	1.44
Mozax (5 mg/tab)	5.0	4.96 ± 0.07	99.20	4.94 ± 0.05	98.80	0.367	1.96
	10.0	9.93 ± 0.08	99.30	9.95 ± 0.06	99.50	0.396	1.77
	15.0	14.98 ± 0.05	99.86	14.97 ± 0.08	99.80	0.167	2.56
Mosid (5 mg/tab)	5.0	4.99 ± 0.04	99.80	4.97 ± 0.05	99.40	0.493	1.56
	10.0	9.97 ± 0.07	99.70	9.98 ± 0.10	99.80	0.129	2.04
	15.0	14.93 ± 0.11	99.53	14.95 ± 0.13	99.66	0.185	1.39
Moza (5 mg/tab)	5.0	5.04 ± 0.06	100.80	5.00 ± 0.08	100.00	0.632	1.77
	10.0	9.96 ± 0.10	99.60	9.97 ± 0.08	99.70	0.123	1.56
	15.0	14.94 ± 0.09	99.60	14.91 ± 0.06	99.53	0.438	2.25
Mozasef (5 mg/tab)	5.0	4.97 ± 0.06	99.40	4.96 ± 0.05	99.20	0.202	1.44
	10.0	9.86 ± 0.04	98.60	9.88 ± 0.05	98.80	0.493	1.56
	15.0	14.84 ± 0.08	98.93	14.79 ± 0.06	98.60	0.790	1.77

a. Mean(SMP-Samples) ± standard deviation(n=5)

b. Tabulated t-value for 8 degree of freedom at P(0.95) is 2.306.

c. Tabulated F-value for (4,4) degree of freedom at P(0.95) is 6.39.

REFERENCES

- [1] Kanaizumi, T. Nakano, H. Matsui, Y. Ishikawa, H. Shimizu, R. Park, S. and Kuriya, N., *Eur. J. Clin. Pharmacol.*, 41, 335-337, 1991.
- [2] Goh, K. L., Chang, C. S., Fock, K. M., Ke, M., Park, H. J. and Lam, S. K., *J. Gastroenterol. Hepatol.*, 15 (3), 230-238, 2000.
- [3] Carisson, L., Amos, J. G. and Anderson, B., *J. Pharmacol. Exp. Therapeutics*, 282, 220-227, 1997.
- [4] Karasawa, T., Yoshida, N., Furukawa, K., Omoya, H. and Ito, T., *Eur. J. Pharmacol.*, 183, 2181-2186, 1990.
- [5] Yoshida, N., Ito, T., Furukawa, K. and Ito, Z., *J. Pharm. Exp. Ther.* 257, 781-787, 1991.
- [6] Rajput, S. J., Sankalia, M. G. and Patel, F. T., *Indian J. Pharm. Sci.*, 67 (5), 582-585, 2005.
- [7] Krishnaiah, Y. S. R., Murthy, T. K., Sankar, D. G. and Satyanarayana, V., *Anal. Sci.*, 18, 1269-1274, 2002.
- [8] Krishnaiah, Y. S. R., Murthy, T. K., Sankar, D. G. and Satyanarayana, V., *Pharmazie*, 57 (12), 814-816, 2002.
- [9] Rao, R. N., Nagaraju, D., Alvi, S. N. and Bhirud, S. B., *J. Pharm. Biomed. Anal.*, 36 (4), 759-767, 2004.

- [10] Kuchekar, B. S., Adagale, V., Nagar, M. and Bhise, S. B., *Indian J. Pharm. Sci.*, 65, 85, 2003.
- [11] Prabhakar, B. K., Shobha, M. and Raju, S. A., *J. Indian Council Chem.*, 20, 42, 2003.
- [12] Prabhakar, B. K., Shobha, M. and Raju, S. A., *Asian J Chem.*, 15, 1081, 2003.
- [13] Raju, S. A. and Shobha, M., *Asian J Chem.*, 15, 529-534, 2003.
- [14] Revanasiddappa, H. D. and Veena, M. A., *Ecl. Quim.*, 32 (4), 71-75, 2007.

طريقة مبسطة للتحليل الطيفي لمادة (MOSAPRIDE) باستخدام طريقة تكوين أصباغ الآزو

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الملخص:

هذه الدراسة تصف طريقة مبسطة للتحليل الطيفي لمادة (mosapride). تعتمد هذه الطريقة على تفاعل مادة (mosapride) مع (nitrite) لتكوين أيون (diazoniun) والذي يتحد مع (acetylacetone) أو مادة (ethyl-acetoacetate) في الوسط القاعدي لتكوين أصباغ الآزو، والتي يتم إمتصاصها على طول موجي ٤٠٢ نانوميتر أو ٤٢٢ نانوميتر.

هذه الطريقة تتبع قانون بير في مجال تركيز يقع بين ٤,٠ و ١٣,٥ (mg/ml) لتفاعل (mosapride) مع (acetylacetone) ومجال تركيز يقع بين ٠,٥ و ١٧,٠ (mg/ml) لتفاعل (mosapride) مع (ethyl-acetoacetate).

لقد وجد أن قيمة الإمتصاصية المولارية لأصباغ الآزو الناتجة من تفاعل (mosapride) مع (acetylacetone) هي (١,٠٢٦ × ١٠^٤ لتر/مول.سم) وقيمة حساسية ساندل لهذا التفاعل بلغت ٤,٤٨٤ × ١٠^{-٣} مايكروغرام لكل سم^٢، ومن ناحية أخرى تم التوصل إلى أن قيمة الإمتصاص المولارية لأصباغ الآزو الناتجة من تفاعل (mosapride) مع (ethyl-acetoacetate) هي (١,٠٩٧ × ١٠^٤ لتر/مول.سم) وقيمة حساسية ساندل لهذا التفاعل بلغت ٤,١٩٣ × ١٠^{-٣} مايكروغرام لكل سم^٢. إن أصباغ آزو المتكونه في كلا الحالتين كانتا مستقرة لمدة ٨ ساعات. في هذه الدراسة تم تحديد أفضل الظروف للتفاعل والعوامل التحليلية المؤثرة وتم تطبيق هذه الطريقة بنجاح لتحديد مادة (mosapride) في العينات الدوائية.