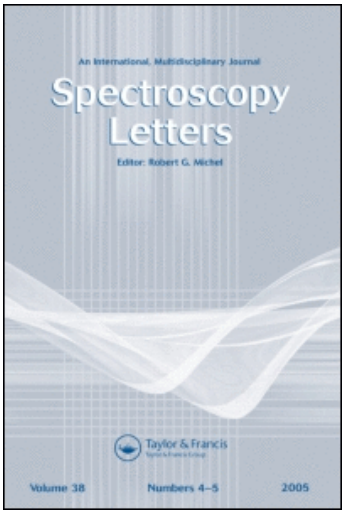

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Sequential Injection Spectrophotometric Determination of Metoclopramide in Pharmaceutical Preparations

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Abstract: In this work, a spectrophotometric methodology for the determination of metoclopramide in pharmaceutical preparations is proposed. The approach was based on the reaction of metoclopramide with Folin–Ciocalteu reagent and was implemented on a sequential injection analysis automated flow system. An in-line mixing chamber was used to promote reaction zone homogenization, to increase reaction time, and to attenuate the high refractive index gradient generated by the utilization of concentrated solutions, thus enabling the minimization of the Schlieren effect. Linear calibration plots for metoclopramide concentrations up to 100 mg L^{-1} were obtained, with good precision (RSD $< 3\%$, $n = 10$). The detection limit was 2.0 mg L^{-1} and the quantification limit was 6.8 mg L^{-1}.

Keywords: Folin-Ciocalteu reagent, metoclopramide, sequential injection analysis, spectrophotometry

INTRODUCTION

Metoclopramide hydrochloride (MCP), 4-amino-5-chloro-*N*-2-(diethylamino)-2-methoxybenzamide hydrochloride (Fig. 1), is a substituted

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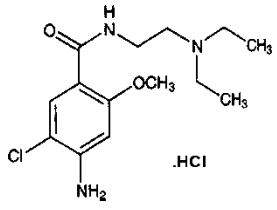

Figure 1. Chemical structure of metoclopramide hydrochloride.

benzamide used for its prokinetic and antiemetic properties in disorders of decreased gastrointestinal motility such as gastroparesis and ileus, as well as in gastrooesophageal reflux disease, dyspepsia, nausea and vomiting, and for the prevention of cancer chemotherapy–induced emesis.[1]

The wide use of this drug has prompted the development of several analytical methods for its determination including liquid chromatography,[2,5] gas chromatography,[6] potentiometry,[7,8] voltammetry,[9,10] amperometry,[11] chemiluminescence,[12,13] and fluorimetry.[14] Spectrophotometry has been the most widely used technique for metoclopramide determination, especially for the analysis of pharmaceutical preparations. The approaches included the formation of charge-transfer complexes using the chloranil and bromanil[15] reaction with *p*-dimethylaminocinnamaldehyde[16] and diazo coupling with benzoylacetone in alkaline medium.[17] The official methods of the United States Pharmacopeia (USP)[18] for metoclopramide hydrochloride determination are based on a nonaqueous potentiometric titration (raw material) and HPLC procedures (pharmaceutical formulations), which are either laborious and time-consuming or require expensive equipment and skillful operators.

The automation of wet-chemical assays to make them faster, more efficient, and more environmentally friendly is a priority in routine and research pharmaceutical laboratories. The usefulness and compatibility exhibited by continuous flow techniques make them ideal tools to fulfill these requirements. A flow injection (FIA) procedure was proposed for MCP determination involving derivatization by the Bratton–Marshall method and spectrophotometric detection.[19] A similar FIA assembly was used in a chemiluminometric assay using the $[Ru(dipy)_3^{2+}]$/permanganate system.[13] These systems enable the determination of metoclopramide between 0.5 and 85 mg L^{-1} and 0.06 and 60 mg L^{-1}, respectively. However, because of the continuous addition of reagents, these systems generate 2 to 5 mL of reagent effluents per determination and employ reagents, such us cadmium and formaldehyde, which can be considered toxic and non–environmentally friendly. Moreover, these procedures lack operational autonomy and are more susceptible to errors because they resort to a rotary valve for the manual insertion of the sample.

Sequential injection analysis (SIA)[20] systems ensure an automation level higher than the one attained with conventional FIA manifolds.[21] In effect, the characteristic multiposition selection valve provides a high operational

versatility and a convenient means of selecting assorted reagents, which allows an easier automation of complex reactions and the reduction of operator input, reagents consumption, and analysis time. Moreover, SIA makes use of a simple, easily configured and robust single-channel manifold, which enables the use of the same manifold for the implementation of a wide range of assays.

The Folin–Ciocalteu reagent (FCR) is a chromogenic agent that has been extensively used in the determination of a number of compounds exhibiting reducing properties and particularly in the determination of phenols.[22,23] The reagent consists of a mixture of phosphomolybdic acid and phosphotungstic acid that are reduced in alkaline medium resulting in the formation of a blue color.

The main goal of this work was the development of a rapid, accurate, easily configured and operated automated analytical procedure for the determination of metoclopramide in pharmaceutical preparations, which was achieved by implementation of a novel spectrophotometric method based on reaction of metoclopramide with FCR. In order to increase the reaction time without affecting sensitivity, the usual reaction coil was replaced by a mixing chamber where the sample zone was halted during a predetermined time interval for reaction development. This way, a faster sample/reagent mixing was achieved while sample dispersion was minimized. Moreover, it allows the attenuation of the refractive index gradients and therefore the Schlieren effect.

MATERIALS AND METHODS

Instrumentation

Spectrophotometric measurements (at 760 nm) were done with a Jenway 6300 spectrophotometer equipped with an 18-μL internal volume, 10-mm optical path flow-cell (Hellma, Müllheim, Balden, Germany).

The SIA analytical manifold (Fig. 2) comprised a Gilson Minipuls 3 peristaltic pump, equipped with a 1.2-mm-i.d. PVC pumping tube and a 10-port multiposition Vici Valco selection valve.

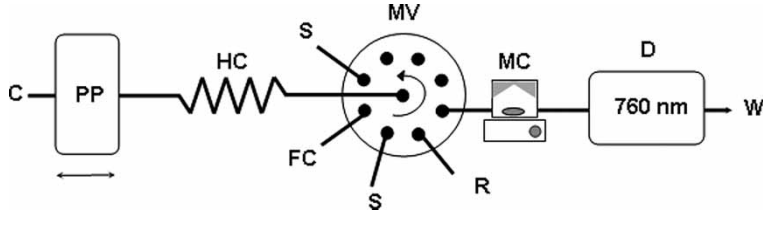

Figure 2. Configuration of the sequential injection analytical manifold: PP, peristaltic pump; C, carrier (water); HC, holding coil; MV, multiposition valve; MC, mixing chamber; D, spectrophotometric detector; W, waste; FC, Folin–Ciocalteu reagent 7% (v/v); R, Na_2CO_3 7% (w/v); S, standard or sample.

All flow lines were made of 0.8-mm-i.d PTFE tubing. A home-made Perspex mixing chamber, with an inner volume of ca. 330 μL (including a magnetic stirrer), was connected to one of the inlets of the selection valve.

Control of the analytical system as well as analytical signal acquisition and processing was accomplished by means of a Pentium-based microcomputer and software developed using Quick-Basic 4.5. The modules of the SIA system were connected to the computer through an Advantech LABcard PCL 711B interface card.

A Kipp & Zonen BD 111 strip chart recorder was used to record the analytical signals when they were not being acquired by the computer.

Sample analysis according to the reference method recommended by the *United States Pharmacopeia* (USP 24)[18] was done in a Merck Hitachi Lachrom Liquid Chromatograph, equipped with an L-7455 diode array detector, an L-7100 pump, and a D-7000 interface card.

Reagents

All chemicals used were of analytical reagent grade and doubly deionized water (conductivity <0.1 μS cm^{-1}) was used throughout.

A 7% (v/v) Folin–Ciocalteu solution (Sigma) was prepared daily by appropriate dilution of the commercial stock solution and kept in the refrigerator. The commercial stock solution was kept in the refrigerator and showed no alteration during all the work.

A 7% (w/v) Na_2CO_3 solution was prepared in boiled, deionized water.

A 1.0 g L^{-1} metoclopramide hydrochloride (Sigma) stock solution was prepared in deionized water. This solution was kept in the refrigerator and protected from light as the drug is affected by light and heat. Working standards were daily prepared by appropriate dilution.

The sample solutions analyzed (Primpéran oral solution, 1 mg mL^{-1}; Primpéran tablets, 10 mg; Primpéran injectable form, 5 mg mL^{-1}) were commercially available. Tablet samples were prepared by dissolving the required amounts of powdered pharmaceutical preparations in water. For each assay, 20 tablets were weighed and a mean content of a single tablet was estimated in order to prepare a solution with an approximately known amount of metoclopramide. Samples of the oral and injectable solutions were prepared by dilution with water.

Flow Procedure

The analytical cycle for the determination of metoclopramide by the proposed flow method included seven steps (Table 1): it began (steps 1, 2, 3) with the sequential aspiration into the holding coil of 60 μL of Folin–Ciocalteu

Table 1. Analytical operating sequence for metoclopramide determination

Step	Position	Time (s)	Flow rate (mL min^{-1})	Description
1	1	3.7	1.0	Aspiration of 67.5 μL of sample
2	2	3.3	1.0	Aspiration of 60.0 μL of Folin—Ciocalteu reagent
3	3	3.7	1.0	Aspiration of 67.5 μL of sample
4	4	3.0	1.0	Aspiration of 55 μL of Na_2CO_3
5	5	8.0	1.0	Propulsion to the mixing chamber
6	5	50	—	Stop period in the mixing chamber
7	5	110	0.8	Propulsion of the reaction zone to the detector

reagent sandwiched between two aliquots of 67.5 μL of sample, which enabled the establishment of two reaction interfaces that favored reaction development. Subsequently, at step 4, an aliquot of 55 μL of Na_2CO_3 was aspirated to the holding coil. Afterwards, the direction of the flowing stream was reversed, promoting the mutual interdispersion of neighboring sample/reagent zones, and the reaction zone was propelled into the mixing chamber (step 5), which was placed between the selection valve and the detector to provide faster homogenization. After a stop period of 50 s (step 6) for reaction development, the carrier swept the content of the mixing chamber toward the detector at a flow rate of 0.80 mL min^{-1} (step 7). The transient signal from the detector was recorded as a peak, whose height was proportional to metoclopramide concentration in the sample. One sample cycle took about 180 s corresponding with a sample frequency of about 20 samples/hour.

Reference Procedure

Aiming at evaluating the accuracy of the results, the available metoclopramide formulations were analyzed according to *United States Pharmacopeia* methods.[18] These methods depended on the type of pharmaceutical form under analysis. For metoclopramide raw material, it involved a nonaqueous potentiometric titration in glacial acetic acid with 0.1 mol L^{-1} perchloric acid (in glacial acetic acid). The chromatographic method with UV detection ($\lambda = 215$ nm) for metoclopramide oral solutions, tablets, and injectable solutions was done using a column packed with a octadecyl silane stationary phase (Waters Symmetry C_{18}, 150 × 3.9 mm i.d., 5-μm particle diameter) and a mixture of acetonitrile and acetate buffer (pH = 6.5) as the mobile phase with a flow rate of about 1.5 mL min^{-1}.

RESULTS AND DISCUSSION

When carrying out preliminary batch experiments, it was observed that metoclopramide reacted with Folin–Ciocalteu reagent producing colored reaction products with maximum absorbance at 760 nm. It was also verified that the reaction was enhanced in alkaline conditions. Because the FCR was prepared in acid medium, it was necessary to use an alkaline solution to adjust the pH of the reaction zone. The evaluation of several alkalis revealed that NaOH and Na_2CO_3 yielded the best results. However, the precision of the analytical signals obtained with sodium carbonate was higher than attained with sodium hydroxide. Furthermore, the detrimental Schlieren effect arising from the establishment of a marked refraction index gradient was more pronounced when NaOH was used. These results motivated the selection of Na_2CO_3 for the subsequent experiments. The evaluation of the most adequate carbonate concentration was a fundamental task; due to the acidic nature of FCR, too low an Na_2CO_3 concentration resulted in the formation of CO_2 bubbles within the tubing, which impaired detection, whereas with too high an Na_2CO_3 concentration, a precipitate was formed. The assessment of Na_2CO_3 concentrations between 1.5% and 7.5% (w/v) showed that the analytical signal increased up to 7% (w/v) and then approached stabilization. The Folin–Ciocalteu reagent was evaluated between 4% and 8% (v/v) and the best results were obtained with a 7% (v/v) concentration.

Analytical Manifold

Because the reaction between metoclopramide and FCR was revealed to be relatively slow, the optimization of the flow system was done by aiming at analytical conditions that promoted a faster reaction zone homogenization and a longer residence time, thus enabling an improved reaction development.

In a preliminary manifold configuration that included a 100-cm reaction coil, the influence of sample and reagent volumes, the sequence for insertion of solutions, and reaction zone establishment were studied in detail, as they play a fundamental role in the sensitivity of the methodology. Due to the viscosity of the solutions, which affected dispersion, instead of inserting the sample as a single volume it was inserted as two identical volumes separated by a single FCR volume. This guaranteed two reaction zone interfaces and faster mixing. However, the limited dispersion was not entirely solved because the utilization of sample volumes larger than 80 μL (two aliquots of 40 μL separated by 40 μL of FCR) yielded two peaks. Moreover, the utilization of concentrated alkaline solutions generated pronounced refractive index gradients that restrained the accuracy, reproducibility, and sensitivity of the analysis, due to the Schlieren effect,[24] which situation was even more problematic in the characteristic limited dispersion conditions of a sequential injection single-channel manifold. Besides the

inadequate mixing, the 100-cm reactor did not provide an adequate residence time for reaction development due to the low reaction kinetics. The evaluation of a longer reactor (200 cm) did not show a significant improvement of sensitivity. An expeditious alternative to increase reaction time is the utilization of a stopped flow strategy. However, considering that dispersion of the sample zone ceases when the carrier stream stops, the mixing of sample and reagents should be either sufficient to promote reaction development when the flow is halted, or maintained during the stopping interval. Owing to the poor dispersion, the second alternative was preferred, and the reaction coil, which was initially placed between the multiposition valve and the detector, was replaced by a ca. 330 μL internal volume mixing chamber (corresponding with the internal volume of a 66-cm reactor, 0.8-mm i.d.), which enabled the incremental mixing of sample/reagent and longer residence time, while providing a suitable sampling rate. Also, along with the sample volume, the FCR and Na_2CO_3 volumes played an important role in terms of system performance and efficiency. The influence of FCR volume was evaluated between 40 and 80 μL. It was observed that the analytical signal increased with FCR volumes up to 60 μL and then approached stabilization. Na_2CO_3 volumes were studied between 15 and 75 μL and an increased sensitivity was obtained with 55 μL, which indicated that this value provided the most adequate pH for reaction development in the mixing chamber.

In a conventional sequential injection system, reaction coil length and flow rate have a profound effect on sample/reagent mixing and reaction time, therefore on sensitivity and sampling rate. However, their impact was minimized due to the introduction of the mixing chamber. With this configuration, it was possible to enhance the reaction zone, both by increasing sample and FCR volumes, while ensuring an adequate homogenization. Which was mostly determined by the time interval during which the reaction zone was halted within the mixing chamber. By evaluating various stop intervals (0 to 120 s), it was verified that analytical signal magnitude increased with stop interval although sampling rate decreased. As a compromise between sensitivity, reproducibility, and sample throughput, a sample volume of 135 μL (two zones of 67.5 μL separated by a 60-μL zone of FCR), a 55-μL zone of Na_2CO_3, and a stop interval (in the mixing chamber) of 50 s were selected for the analysis.

Concerning the reaction mechanism, we believe that metoclopramide reduces FCR with the formation of a blue chromogen with a λ_{max} at 760 nm, in a process similar to the Lowry method for protein determination.[25]

Analysis of Pharmaceutical Preparations

After optimization of the system and reagent concentrations (7% w/v Na_2CO_3 and 7% v/v FCR) and using the analytical sequence displayed in Table 1, a linear working range of up to 100 mg L^{-1}, was obtained.

The calibration curve was represented by the equation $A = 0.0029\ (\pm 0.0001)\ C + 0.005\ (\pm 0.005)$, where A represents peak height (in cm), C represents metoclopramide concentration (in mg L^{-1}), and the values in parentheses refer to the standard errors. The correlation coefficient was 0.998 ($n = 6$). The limit of detection as defined by IUPAC, $C_{LOD} = 3\ S_b/m$ (where S_b is the standard deviation of blank and m is the slope of the calibration curve) was estimated to be 2 mg L^{-1}. The relative standard deviation (RSD) for two distinct metoclopramide concentrations, 27.5 and 90 mg L^{-1}, was 3% and 1.5%, respectively, based on 15 replicated measurements.

All reducing species are susceptible to interfere in the metoclopramide reaction with FCR. In this sense, the selectivity of the proposed methodology was evaluated by monitoring a 20 mg L^{-1} metoclopramide standard solution in the presence of increasing concentrations of other formulation components (sucrose, lactose, talc, starch, and magnesium stearate). An excipient was considered as noninterfering if the analytical signal variation was lower than 3%, with respect to the signal obtained in its absence. It was observed that the analytical response was not significantly different from the one obtained with metoclopramide alone up to an excipient/metoclopramide molar ratio of 100.

The performance of the methodology was tested in the determination of metoclopramide in various pharmaceutical forms (tablets, oral solution, and injectable solution). The results obtained agreed with those furnished by the reference procedure[18] with a relative error (RE) lower than 4.0% (Table 2). These results were confirmed through Student's t-test, which showed that there was no significant difference, at the 95% confidence level, between the proposed and the reference procedures (calculated t-value = 0.303; tabulated t-value = 4.30). Sampling rate was about 20 samples per hour.

Table 2. Results obtained in the determination of metoclopramide by the proposed (SIA) and the reference method (HPLC)

		Amount found (mg/formulation)		
Pharmaceutical preparation	Amount declared (mg/formulation)	SIA[a]	Reference method[a]	RE (%)[b]
Primpéran (tablets)	10 mg	10.05 ± 0.04	9.97 ± 0.05	+0.8
Primpéran(injectable)	10 mg	9.96 ± 0.01	9.92 ± 0.03	+4.0
Primpéran (oral solution)	2.6 mg	2.85 ± 0.03	2.90 ± 0.04	−1.7

[a]Mean ± standard deviation (obtained after threefold sample processing).

[b]Relative error (RE) of the developed sequential injection analysis (SIA) methodology with respect to the reference method.

The system was stable and its performance remained unaltered throughout the assessment of the procedure accuracy. No baseline drift was observed.

CONCLUSIONS

The automated determination of metoclopramide by sequential injection analysis using the Folin–Ciocalteu reagent, and spectrophotometric detection of the products, exhibited adequate sensitivity, selectivity, reproducibility, and accuracy to be used as an analytical alternative for the determination of this drug in raw materials and pharmaceutical preparations.

The approach is simple and provides a good sampling frequency, a wide range of application with low sample and reagent consumption, while at the same time it ensures a high automation level that minimizes operator intervention and the occurrence of errors, which makes it suitable for application for routine analysis and in quality-control laboratories. The inclusion of a mixing chamber in an SIA manifold was revealed to be an expeditious alternative to the commonly used reaction coil, as it ensures enhanced sample/reagent mixing and provides the means for increased reaction development when a stopped-flow approach is implemented.

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