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DEVELOPMENT AND VALIDATION OF RP-LC AND UV SPECTROPHOTOMETRIC METHODS TO ASSAY BROMOPRIDE IN ORAL AND INJECTABLE SOLUTIONS

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A reversed-phase liquid chromatographic (LC) and ultraviolet (UV) spectrophotometric methods were developed and validated for the assay of bromopride in oral and injectable solutions. The methods were validated according to ICH guideline. Both methods were linear in the range between 5-25 µg mL^{-1} (y = 41837x – 5103.4, r = 0.9996 and y = 0.0284x – 0.0351, r = 1, respectively). The statistical analysis showed no significant difference between the results obtained by the two methods. The proposed methods were found to be simple, rapid, precise, accurate, and sensitive. The LC and UV methods can be used in the routine quantitative analysis of bromopride in oral and injectable solutions.

Keywords: bromopride; reversed-phase liquid chromatography; ultraviolet spectrophotometry.

INTRODUCTION

Bromopride (4-amino-5-bromo-*N*-(2-diethylaminoethyl)-2-methoxybenzamide) (Figure 1), a white crystalline powder, is the bromo-analogue of metoclopramide, being both benzamide derivatives. Bromopride stimulates the motility of the gastrointestinal tract, enhances gastric emptying rate, and presents antiemetic properties. Bromopride is used to treat a variety of gastrointestinal disorders. The main action of bromopride is related to blockade of dopamine 2-receptors in the central nervous system and gastrointestinal tract. Similar to other benzamide derivatives, the gastrointestinal tract stimulation by bromopride appears to be mediated at least in part by its indirect cholinergic activity.[1]
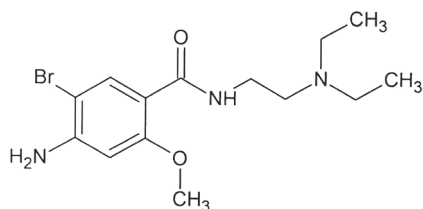

Figure 1. *Chemical structure of bromopride*

Some techniques have been used to determine bromopride in different biological matrices. Such methods include liquid chromatography-electrospray tandem mass spectrometry,[2] high-performance liquid chromatography with UV detection,[3-5] thin-layer chromatography and gas chromatography.[6] The pharmacopoeia does not present methods for the assay of bromopride in oral and injectable solutions. To date, there are no previous reports focused on the development and validation of analytical methods for the quantitative analysis of bromopride as active substance in pharmaceutical dosage form.

Considering the lack of methods available to assay bromopride in pharmaceutical formulations, this work describes the development and validation of reversed-phase liquid chromatographic (RP-LC) and UV spectrophotometric methods for the determination of bromopride in pharmaceutical dosage forms, establishing procedures for their

quality control. Both methods have been applied for the quantitative analysis of bromopride in oral and injectable solutions.

EXPERIMENTAL

Chemicals

Bromopride reference substance (assigned purity 99.11%) was purchased from DEG Importadora de Produtos Químicos Ltda (São Paulo, Brazil). Digesan® (Sanofi-Synthelabo), oral and injectable solutions containing 4 and 5 mg mL^{-1} of bromopride, respectively, was obtained from the market (Batches 6V2619 and 6Y002, respectively). Gradient-grade LiChrosolv acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Monobasic sodium phosphate (KH_2PO_4) and triethylamine (TEA) were also obtained from Merck. Hydrochloric acid was obtained from Tedia (Fairfield, USA). Water was purified with WaterPro™ PS, Labconco system (MO, USA). Bromopride oral solution was labeled to contain the following excipients: sodium metabisulfite, methylparaben, propylparaben, sodium saccharin, disodium edetate, hydrochloric acid and water. Bromopride injectable solution was labeled to contain the following excipients: hydrochloric acid, sodium chloride and water.

Instrumentation and analytical conditions

The LC method was performed on a Shimadzu LC system (Kyoto, Japan) equipped with a CBM-20A system controller, LC-20AT pump, DGU-20A$_5$ degasser, SIL-20A auto-sampler and SPD-M20A photodiode array detector (PAD). Analytical separation was performed on a Phenomenex (Torrance, USA) Gemini C$_{18}$ column (150 × 4.6 mm i.d., 5 µm particle size) coupled to a C$_{18}$ guard column (40 × 3.0 mm i.d., 4 µm). The isocratic mobile phase was a mixture of 10 mM KH_2PO_4 + 0.1% TEA (pH 3.0 adjusted with phosphoric acid): acetonitrile (80:20, v/v), performed at room temperature (25 ± 1 °C), which was pumped at a flow-rate of 1.0 mL min^{-1}. This was filtered through a 0.45 µm nylon membrane filter (Sartorius, Germany) and degassed before used. The injection volume was 20 µL (variable injection volume valve – 0.1 to 100 µL). The detection was achieved with a PAD at 310 nm (Figure 2).
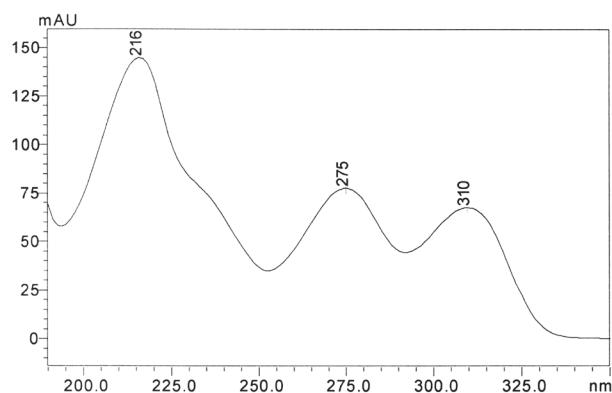
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Figure 2. *UV spectra of bromopride reference standard by HPLC-DAD at 15 μg mL⁻¹*

The UV spectrophotometric method for quantitative assay of bromopride in oral and injectable solutions was performed on a Shimadzu UV 1601 PC spectrophotometer (Kyoto, Japan) equipped with 1 cm quartz cells with detection at 310 nm and 0.1 M hydrochloric acid was used as diluent.

Preparation of reference solution

LC method

An accurately weighed 10 mg portion of bromopride reference standard was transferred to a 100 mL volumetric flask and dissolved in methanol (final concentration = 100 μg mL⁻¹). This solution was diluted with ultra-pure water to give five standard solutions with different concentrations of bromopride (5.0, 10.0, 15.0, 20.0, and 25.0 μg mL⁻¹), which were used in the analytical curve for the linearity evaluation.

UV method

Stock standard solution (100 μg mL⁻¹) was prepared by dissolving 10.0 mg bromopride reference standard in 100.0 mL of 0.1 M hydrochloric acid. The stock standard solution was diluted with 0.1 M hydrochloric acid to give five standard solutions with different concentrations of bromopride (5.0, 10.0, 15.0, 20.0, and 25.0 μg mL⁻¹), which were used in the analytical curve for the linearity evaluation.

Preparation of sample solution

LC method

An amount equivalent to 10 mg of bromopride, present in oral and injectable solutions, was transferred to a 100 mL volumetric flask and diluted with methanol to obtain a concentration of 100 μg mL⁻¹. These solutions were diluted with ultra-pure water to obtain final concentrations of 15 μg mL⁻¹ and filtered through a 0.45 μm nylon membrane filter (Sartorius, Germany) before LC analysis.

UV method

An amount equivalent to 10 mg of bromopride, present in oral and injectable solutions, was transferred to a 100 mL volumetric flask and diluted with 0.1 M HCl to obtain concentrations of 100 μg mL⁻¹. These samples were diluted with 0.1 M HCl to obtain a final concentration of 15 μg mL⁻¹ before analysis on the spectrophotometer.

Method validation

Validation was carried out assessing the following parameters: linearity, range, specificity, precision, accuracy, detection and quantification

limits, according to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures.[7,8] The system suitability test was also carried out to evaluate the reproducibility of the analytical system, using five replicate injections of a reference solution. The parameters measured were injection repeatability, retention time, theoretical plates, peak symmetry, and capacity factor.

Linearity

The analytical curves were obtained with five concentrations of reference solution in the range of 5-25 μg mL⁻¹ for the spectrophotometric and LC methods. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis by the least-square regression method, which was used to calculate the correlation coefficient, y-intercept and slope of the regression line.

Precision

The precision of the procedures was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated assaying six determinations at the same concentration (15 μg mL⁻¹), during the same day, under the same experimental conditions. Intermediate precision was analyzed comparing the assays in six determinations at the same concentration (15 μg mL⁻¹) during three different days. Precision (repeatability and intermediate precision) was expressed as relative standard deviation (RSD%).

Accuracy

Accuracy was evaluated assaying samples of a known concentration (7.5 μg mL⁻¹) spiked with three different concentrations of standard solution (3.75, 7.50 and 11.25 μg mL⁻¹), at three different levels (lower, medium and upper concentration), giving sample solutions with concentrations of 11.25, 15.00 and 18.75 μg mL⁻¹. The accuracy was calculated as the percentage of the drug recovered from the formulation and also expressed as the relative standard deviation (RSD) between the measurements.

Specificity

The specificity of the LC method was evaluated through the analysis of a placebo solution. The placebo consisted of all the excipients without the active ingredient. For both methods, the interference from the formulation matrix was evaluated. The system response was examined for the presence of interference or overlaps with bromopride responses.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope using three independent analytical curves, as defined by ICH.[7] LOD and LOQ were calculated as 3.3 and 10σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Method comparison

The chromatographic and spectrophotometric methods to assay bromopride in pharmaceutical formulations proposed in our study were compared using statistical analysis by Student's t-test. Six samples of each pharmaceutical dosage forms were analyzed by both methods (LC and UV).

RESULTS AND DISCUSSION

LC method

A simple RP-LC method was developed to assay bromopride in pharmaceutical formulations. The method was optimized to provide a good separation of the components (acceptable theoretical plates and resolution between peaks) with a sufficient sensitivity and suitable peak symmetry (peak tailing factor < 2)[9] in a short run. For this purpose, analytical column, solvent selection, mobile phase composition, flow-rate, and detector wavelength were studied. The chromatographic separation was achieved using a reversed-phase C_{18} column because it is suitable to analyze bromopride with adequate resolution and symmetrical peak shapes. The chromatograms of bromopride reference standard, samples, and placebo solutions are demonstrated in Figure 3. Analyzing this figure, it can be observed that the proposed LC method is specific since no interfering peaks were observed in the placebo samples. In order to confirm this absence of interference, a peak-purity evaluation using the photodiode-array (PDA) was carried out. These analyses showed that no impurities and/or excipients were co-eluting with the bromopride peak. In addition, the retention time (5.1 min) allows rapid determination of the drug, which is important for routine analysis. Besides the presence of methyl and propyl parabens in the oral solutions, as preservative, the corresponding peaks were not observed in the chromatograms due to their lack of UV absorption at 310 nm. Methyl and propyl parabens presented peak maxima around 254 nm in acid solution.[10]
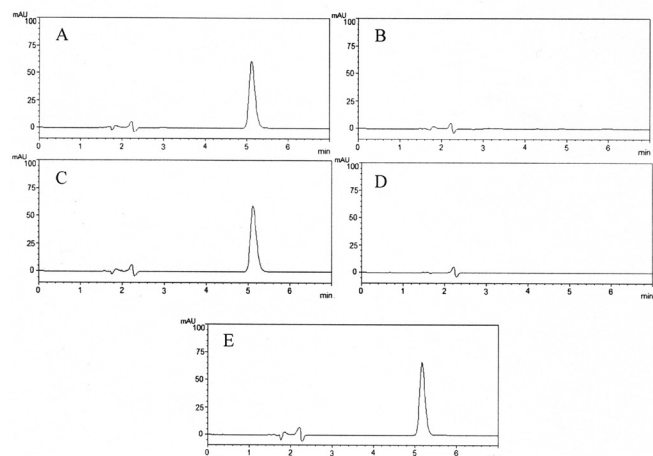

Figure 3. *Chromatograms of bromopride in oral and injectable solution at 15 μg mL⁻¹ (A and C), their respective placebos (B and D) and reference standard solution (E) at 15 μg mL⁻¹*

The analytical curves for bromopride were constructed by plotting concentration versus peak area. A good linearity was observed in the range between 5 and 25 μg mL⁻¹. The linear equation obtained by the least-square method was $y = 41837x - 5103$ (n = 3) showing an adequate correlation coefficient (r = 0.9996). LOD and LOQ were 0.15 and 0.50 μg mL⁻¹, respectively. These low values indicate a good sensitivity of the proposed method. These data were validated by means of ANOVA, showing a linear regression with no deviation from linearity.

The results obtained for precision are presented in Table 1. All RSD values were lower than 1.0%, indicated good intra-day and inter-day precision.[11,12] Regarding the accuracy evaluation, good recoveries (100-102 %) were obtained (Table 2). The percent recovery indicated good accuracy[13] and, consequentially, an agreement between the theoretical value and the real value of concentration.[11] These results demonstrated the adequate precision and accuracy of the developed LC method to assay bromopride in such formulations.

Table 1. Repeatability (intra-day precision) and intermediate precision (inter-day) of oral and injectable solutions determined by the LC method

	Mean ± SD (μg mL⁻¹)	Recovery (%)	RSD (%)
Oral solution			
Intra-day (n = 6)	15.07 ± 0.04	100.50	0.32
Inter-day			
Day 1 (n = 6)	15.08 ± 0.05	100.50	0.33
Day 2 (n = 6)	15.10 ± 0.04	100.67	0.27
Day 3 (n = 6)	15.01 ± 0.01	100.05	0.05
Injectable solution			
Intra-day (n = 6)	15.03 ± 0.03	100.25	0.19
Inter-day			
Day 1 (n = 6)	15.04 ± 0.03	100.26	0.20
Day 2 (n = 6)	15.07 ± 0.04	100.49	0.28
Day 3 (n = 6)	15.05 ± 0.05	100.35	0.34
Mean ± SD (n = 18)	15.05 ± 0.04	100.41	0.30

Table 2. Accuracy of oral and injectable solutions by the LC method

	Added (μg mL⁻¹)	Found (μg mL⁻¹)	Recovery (%)	RSD (%)
Oral solution	3.75	3.78 ± 0.01	100.70	0.30
	7.50	7.51 ± 0.01	100.17	0.11
	11.25	11.27 ± 0.01	100.17	0.08
Injectable solution	3.75	3.76 ± 0.01	100.41	0.24
	7.50	7.51 ± 0.01	100.19	0.16
	11.25	11.28 ± 0.02	100.32	0.17

UV method

The UV method for the assay of bromopride was developed as an alternative technique, considering its rapid and low-cost as well as its simple instrumentation compared with other techniques. Since bromopride is freely soluble in 0.1 M HCl this solution was chosen as diluent for the UV assay. The UV spectra of bromopride reference standard, bromopride oral and injectable solutions are represented in Figure 4. As can be seen in Figure 4, the UV spectra from injectable solution overrides the UV spectra obtained for the reference standard solution. As explained previously, the presence of methyl and propyl parabens in the oral solution did not show any interference on the UV spectra because their lack of absorption at 310 nm.[10]

Good linearity was observed in the 5.0-25.0 μg mL⁻¹ range, as previously observed for the LC method. The linear equation obtained by the least-square method was $y = 0.0284x - 0.0351$ showing an adequate correlation coefficient (r = 1). Data were validated by means of ANOVA, which demonstrated linear regression with no linearity deviation. The LOD and LOQ of the UV method were 0.48 and 1.60 μg mL⁻¹, respectively. Although LOD and LOQ of the UV method were higher than those obtained for the LC method (0.15 and 0.50 μg mL⁻¹, respectively), the results from both methods show their good sensitivity to assay bromopride in the concentration range proposed in our method.

Regarding the evaluation of the precision and accuracy of the UV method (Tables 3 and 4, respectively), the results are similar to those obtained for the LC method. All RSD values were lower than
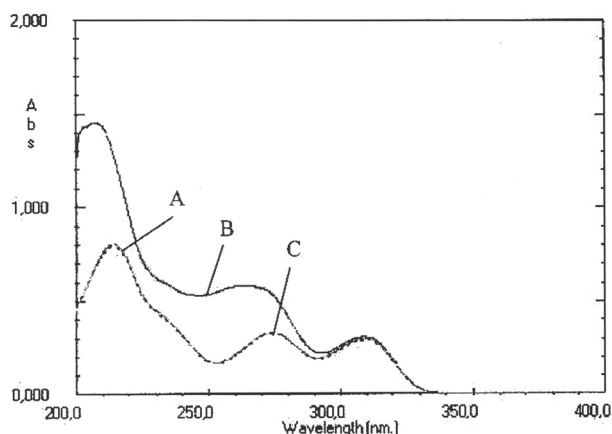
Figure 4. UV spectra of bromopride reference standard (A), bromopride oral (B) and injectable (C) solutions prepared in 0.1 M HCl at 15 µg mL⁻¹

Table 3. Repeatability (intra-day precision) and intermediate precision (inter-day) of oral and injectable solutions determined by the UV method

	Mean ± SD ($\mu g\ mL^{-1}$)	Recovery (%)	RSD (%)
Oral solution			
Intra-day (n = 6)	15.03 ± 0.04	100.25	0.29
Inter-day			
Day 1 (n = 6)	15.04 ± 0.04	100.26	0.30
Day 2 (n = 6)	15.03 ± 0.02	100.18	0.12
Day 3 (n = 6)	15.07 ± 0.03	100.49	0.21
Injectable solution			
Intra-day (n = 6)	15.02 ± 0.02	100.18	0.19
Inter-day			
Day 1 (n = 6)	15.03 ± 0.03	100.18	0.19
Day 2 (n = 6)	15.09 ± 0.02	100.57	0.12
Day 3 (n = 6)	15.05 ± 0.05	100.34	0.32
Mean ± SD (n = 18)	15.05 ± 0.04	100.36	0.26

1.0 % and recoveries were in the range of 100-102%. These results demonstrated adequate precision and accuracy of the UV method.

Comparison between the LC and UV methods

LC and UV methods were compared using Student's t-test. There was no significant difference between the experimental values obtained through the analysis of six samples using the two methods, regardless of the dosage form (p > 0.05). This result shows that the 2 methods are equivalent for the quantitative determination of bromopride in oral or injectable solutions, as showed by some authors for other drugs. [14-17]

CONCLUSIONS

Rapid, specific and reliable LC and UV methods were developed and validated for the assay of bromopride in oral and injectable solutions. Both analytical methods are simple, precise, accurate, sensitive,

Table 4. Accuracy of oral and injectable solutions by the UV method

	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery (%)	RSD (%)
Oral solution	3.75	3.79 ± 0.03	101.05	0.93
	7.50	7.53 ± 0.02	100.41	0.27
	11.25	11.28 ± 0.02	100.30	0.18
Injectable solution	3.75	3.79 ± 0.03	101.14	0.92
	7.50	7.55 ± 0.03	100.62	0.47
	11.25	11.29 ± 0.02	100.36	0.18

and linear in the concentration range of 5-25 µg mL⁻¹, allowing complete interchange, encouraging its application for quality control of bromopride in oral or injectable solutions. In addition, the proposed UV method uses no organic solvent avoiding the formation of dangerous residues promoting benefits to the public health and the environment.

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