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Validation of an UV-Visible spectrophotometry assay method for the determination of chlorpheniramine maleate tablets without prior extraction

Linda Carole Djiambeu CHAWE¹, Nassifatou Koko TITTIKPIA^{1,3*},
Serigne Momar NDIAYE¹, Amadou DIOP¹, Bara NDIAYE¹, Djibril FALL²,
Yérém Mbagnick DIOP¹ and Serigne Omar SARR¹

¹Laboratoire de Chimie Analytique et Bromatologie, Faculté de Médecine, de Pharmacie et d'Odontologie, Université Cheikh Anta DIOP, BP 5005, Dakar-Fann, Sénégal.

²Laboratoire de Chimie Organique et Thérapeutique, Faculté de Médecine, de Pharmacie et d'Odontologie, Université Cheikh Anta Diop de Dakar, BP 5005, Dakar-Fann, Sénégal.

³Département des Sciences Pharmaceutiques (Department of Pharmaceutical Sciences), Faculté des Sciences de la Santé (Faculty of Health Sciences), Université de Lomé (University of Lomé), 01 BP 1515, Lomé 01, Togo.

*Corresponding author; E-mail: knassifa@yahoo.fr; Tel.: 00228 91 83 87 83/00228 91 00 83 80

Received: 08-11-2020

Accepted: 19-02-2021

Published: 28-02-2021

ABSTRACT

Methods proposed by pharmacopeias to check the quality of chlorpheniramine maleate tablets are multi steps methods which involve extraction and present issues with repeatability. An alternative method is proposed with a sensitive, reliable, simple and rapid UV-VISIBLE spectrophotometry method developed for the determination without extraction of chlorpheniramine maleate in tablets. The method was validated using the accuracy profile approach with an accuracy ranging from 99.70 to 100.46%. Analysis was done using 0.25 mol/L sulfuric acid, distilled water, and raw material in a room at 23 °C for 25 to 30 minutes. Chlorpheniramine maleate concentration varied from 0.018 to 0.03 mg/mL. The method was found to be specific with the appearance of the corresponding maxima at 265 nm and a correlation coefficient (R^2) of 0.9993. Limits of detection and quantification were respectively 1.39×10^{-4} and 2.26×10^{-3} mg/mL. The tolerance limits for the different concentration levels (75, 100 and 125%) were respectively: 88.37; 92.74 and 91.62% for lower limits and 111.03; 108.17 and 108.02% for upper limits. It was observed that the tolerance limits were within the limits of acceptability set at 20%. Consequently, the method was declared valid and reliable for routine analysis of chlorpheniramine maleate in tablets containing chlorphenamine as active principle.

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Keywords : Chlorpheniramine maleate, tablets, accuracy profile, assay method without extraction, validation.

INTRODUCTION

Allergy is defined as an overreaction and unwanted defense of the immune system in response to normally harmless substances (allergens). In immunology it is considered to be a hypersensitivity reaction. There are different types classified according to the mechanism and the onset of symptoms (Gali et al., 2008). Many molecules are used in the treatment of allergies and they are named antihistamines. As the name suggests, antihistamines work by blocking the effect of histamine, a protein involved in many allergic reactions. This pharmaceutical class is like all the others, subject to defects, counterfeits or degradations. The circulation of non-compliant drugs represents a permanent threat to public health. Their frequency, which is difficult to quantify, appears to be increasing (around 10% of the world market). This traffic seems to have worsened in recent years and particularly affect developing countries where, around 25% of drugs consumed are of poor quality (WHO, 2017). If it cannot be guaranteed that these drugs actually meet the priority health needs of countries and that their quality, safety and efficacy are at an appropriate level, there is clearly a risk for the health of the population. Their consequences in terms of public health explain that a number of countries, encouraged by the WHO, have set up national laboratories for quality control of medicines. Unfortunately, due to lack of resources, the latter could not check the quality of all pharmaceutical products circulating in the countries (WHO, 2017; Djibrine et al., 2018; Diabaté et al., 2018; Ihaku et al., 2019). In the treatment of allergy, Chlorpheniramine Maleate, also known as Chlorphenamine Maleate is considered by the World Health Organization (list updated in April 2013) as an essential drug thanks to its effectiveness and lower cost and, is distributed worldwide as a generic compound (Hall and Hall, 2014; Djambeu, 2015). Chlorphenamine Maleate is used to relieve allergy symptoms such as watery and itchy eyes, sneezing, runny nose, rash, skin irritation, itching caused by insect bites and other conditions such as chicken pox and

measles. The dosage is less than 24 mg per day in adults, less than 6 mg per day in children aged 2 to 6 years and less than 12 mg per day in children aged 6 to 12 years (Hall EJ and Hall GH, 2014). Unfortunately, this pharmaceutical product is also subject to the risk of counterfeiting. It becomes important to control its quality because it is an essential drug. Methods are available in the American and British Pharmacopoeias to check the quality of chlorphenamine maleate products but they are all "extractive" methods requiring an extraction of the compound prior to its identification and dosage (MHRA 2009; USP 2020). Those methods are also divided into a multitude of difficult easy steps, which could be carried out over a period of 2 hours on average. The methods consume a lot of solvents, most of which are toxic and are all based on the use of High Performance Liquid Chromatography (HPLC). Considering the unavailability of resources and equipment such as HPLC in many national quality control laboratories in developing countries, it is needed to develop an easy method to be performed with readily available equipment. To achieve this aim, this study was realized to develop and validate an easy, simple and inexpensive spectrophotometric method to control quality of chlorpheniramine maleate containing tablets without prior extraction.

MATERIALS AND METHODS

Materials

Reagents

The main compound or active ingredient, chlorpheniramine maleate (CM, Figure 1) and the excipients (sucrose, lactose monohydrate, corn starch and talc) were obtained from Toskani Pharma laboratory (Banjul, Gambia). Sulfuric acid at a concentration of 1 mol/L was obtained from Merck (Darmstadt, Germany) and ultrapure water from a Milli-Q® water purification system (Millipore, Molsheim, France).

Apparatus

A JASCO UV/Vis double beam spectrophotometer (model V-570) with 1 cm quartz cells was used for all spectral measurements.

Methods

Preparation of solvent

Sulfuric acid (0.25 mol/L) was used as a solvent throughout the operation, as a second rinse after a first rinse with ultrapure water and also in the preparation of the samples. To prepare this solvent, 30 mL of sulfuric acid (1mol/L) was poured into 1020 mL of water and the appropriate dilutions were made (USP, 2020).

Operating mode

The sample was prepared by mixing 3 mg of chlorpheniramine maleate with 82 mg of sucrose, 82 mg of lactose monohydrate, 15 mg of corn starch and 6 mg of talc (this composition corresponds to that of a tablet of 3 mg chlorpheniramine maleate available on the markets for patient care). The mixture was put in 50 mL of 0.25 mol/L sulfuric acid, shaken for 5 min, ultrasonicated for 15 min and then filtered. Afterwards, 10 mL of the solution obtained was poured into a 25 mL flask and filled up to 25 mL with the solvent and the absorbance was recorded at the selected wavelength.

The solvent was prepared the same day and all the operations took place in a room at around 23 °C temperature, for 25 to 30 minutes.

Method validation

The method was validated by evaluating the following parameters: selectivity, linearity and range, accuracy, trueness (recovery), limit of detection (LOD) and limit of quantification (LOQ) as specified in the International Conference on Harmonisation protocol (ICH, 2005). The method has been validated using the accuracy profile as previously described (Feinberg, 2007; Frampas et al., 2018; Ibrahim et al., 2019; Tittikpina et al., 2020).

Selectivity/ wavelength selection: Two solutions were prepared with the first solution containing the active ingredient or active principle and the excipients and the second one, the active ingredient or active principle only. The absorption spectra of the two solutions were recorded in the 200-800 nm range and the resulting spectra were overlaid

to determine the optimal wavelength. All the samples were read at this selected wavelength.

Linearity and range: two solutions of concentrations 0.06 and 0.1 mg/mL were prepared following the sample preparing procedure. Standard dilutions were made to obtain the following concentrations: 0.018; 0.022; 0.024; 0.03 and 0.034 mg/mL using a 25 mL flask. The absorption spectrum of each concentration was recorded three times.

Accuracy: it was evaluated by running intra-day and inter-day precision studies. Intra-day precision (repeatability) was performed at 75, 100 and 125% of a selected concentration, with three replicates on the same day. Inter-day precision (intermediate precision) was performed at the same concentrations with 3 replicates and on 3 consecutive days. The relative standard deviation (RSDs) values (RSDR: Relative Standard Deviation of the daily Repeatability and RSDIP: Relative Standard Deviation of the Intermediate Precision) were calculated as previously described (Tittikpina et al., 2020).

Trueness (recovery): it was determined by comparing the concentrations really obtained with the theoretically expected concentrations (R) as previously described (Tittikpina et al., 2020).

Limit of detection (LOD) and limit of quantification (LOQ): the blank solution (containing only the solvent) was prepared and the quartz cell was filled 20 times and submitted one after the other to spectral recording. The values recorded at the selected wavelength made possible to determine the detection and quantification limits using the following formulas:

$$LOD = m_{blank} + 3S_{blank}$$

$$LOQ = m_{blank} + 10S_{blank}$$

With **S** the standard deviation of the blank measurements, **m** mean of the read absorbances, **3** the multiplication factor corresponding to a risk of 0.13% of concluding that the analyte is present and **10** the multiplication factor corresponding to a risk of 0.05% of concluding that the analyte is absent.

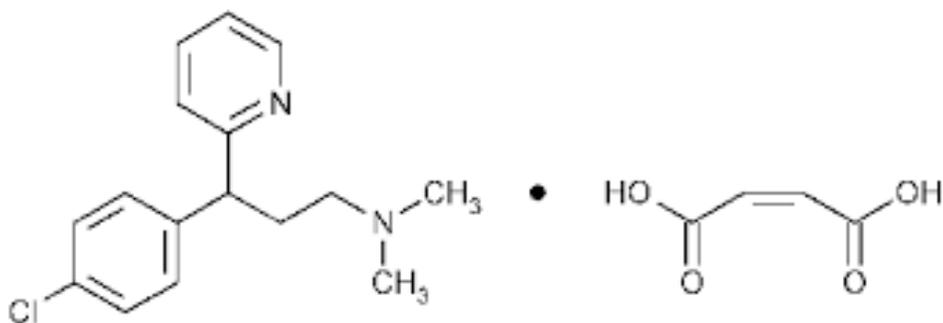


Figure 1: Chlorpheniramine maleate chemical structure.

RESULTS

Selectivity/ wavelength selection

By examining the graphs obtained with the two prepared solutions, it could easily be noticed that the graphs almost fit in each other (Figure 2). It could therefore be concluded that the method is selective. Besides, the maxima obtained at 265 nm was identical to the one used by the United States Pharmacopeia (USP, 2020). 265 nm is consequently the wavelength of choice for this method.

Linearity and Range; Intra-Day and Inter-Day precisions and Trueness; Limit of Detection (LOD) and Limit of Quantification (LOQ)

Linearity was obtained over a concentration range of 0.018 to 0.03 mg/mL with a correlation coefficient R^2 of 0.9993 (Figure 3). RSDs values for intra-day and inter-day precision studies were belonging respectively to the following ranges: 1.51 to 3.07% and 2.07 to 3.07% (Table 1) and recoveries obtained were ranging from 95.13 to 104.34% (Table 2). Finally, the LOD and LOQ recorded are respectively 1.39×10^{-4} and 2.26×10^{-3} mg/mL.

Validation of the method

The validation of the method was performed as previously reported (Tittikpina et al., 2020). Considering the different norms used to perform the validation, the degree of freedom and the risk were established at 6 and 1%, respectively. The inverse Student Test (t) calculated and computed with those parameters gave a value equal to 3.7074. The confidence interval (CI), the upper bound (UB) and lower bound (LB) of the accuracy profile of the method were calculated. The acceptance limit β was set at 20% allowing the computation of the superior and inferior limits of the accuracy profile. The values obtained for the different parameters computed for the establishment of the accuracy profile are summarized in Table 3 and the graph obtained is reported in Figure 4. From the accuracy profile (Figure 4), it could be concluded that the validation of the method could be set on the interval of the concentrations surrounding 0.024 mg/mL (75 to 125%) also corresponding to the linearity interval: 0.018 to 0.03 mg/mL.

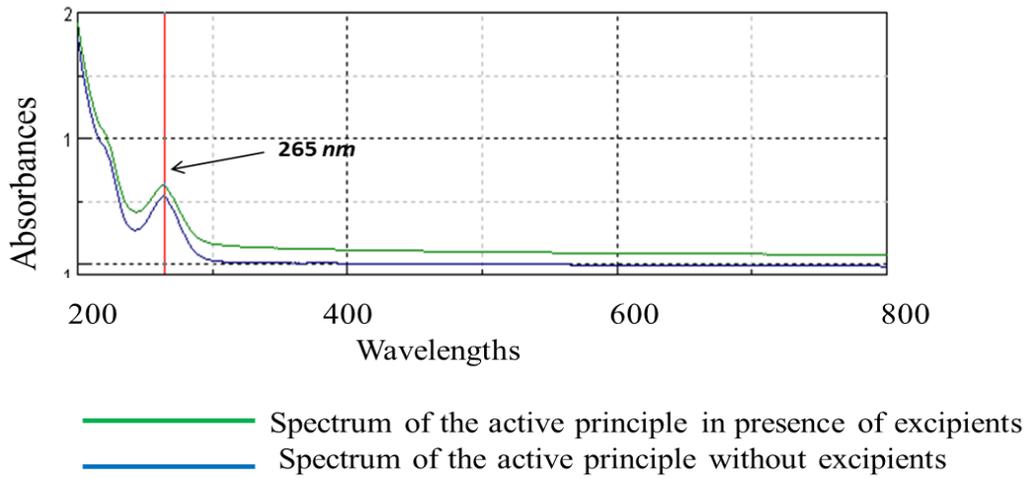


Figure 2: Chlorpheniramine maleate absorption spectrum.

265 nm is the wavelength at which the maximum absorption is observed with the active principle either alone or in presence of excipients.

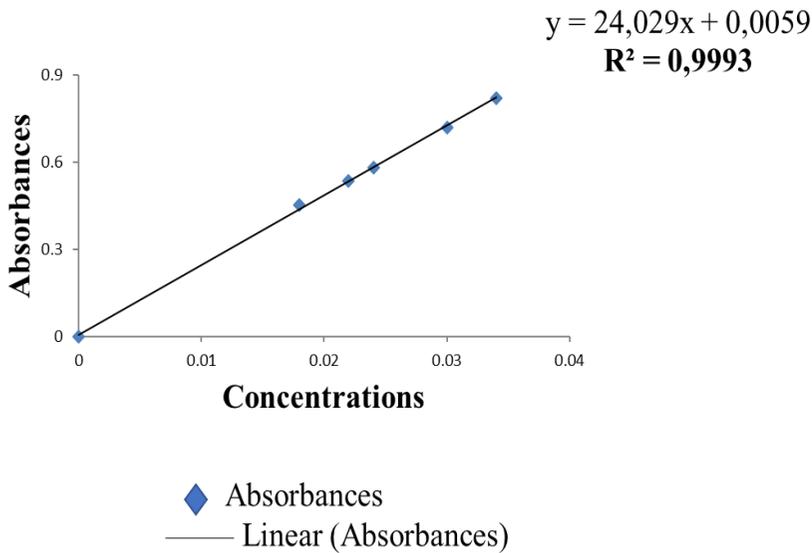


Figure 3: Calibration curve obtained with different concentrations of the samples containing chlorpheniramine as active principle.

R= correlation coefficient. It has a value very close to 1 and shows a linear correlation between concentration and absorbance.

Table 1: Summary of RSDs evaluated for intra-day and inter-day precision studies.

Concentrations (%)	75	100	125
RSD intra-day precision (%)	3.07	1.51	2.22
RSD inter-day precision (%)	3.07	2.07	2.22%

RSD : Relative Standard Deviation.

Table 2: Summary of the percentages of recoveries for the various concentrations (% of 0.024 mg/mL).

Concentration	Replicate 1	Replicate 2	Replicate 3
75%	98.53%	99.27%	102.00%
75%	101.01%	98.35%	95.13%
75%	99.93%	97.95%	105.10%
100%	100.12%	104.34%	97.70%
100%	100.02%	102.67%	99.35%
100%	100.64%	99.64%	99.61%
125%	100.70%	101.79%	101.95%
125%	100.67%	97.33%	102.04%
125%	98.32%	98.21%	97.34%

Table 3: Summary of the accuracy profile.

Conc	Accuracy	RSDR	RSDIP	CI	UB	LB	SL	IL
75%	100.26%	5.30%	5.30%	15.63%	115.89%	84.63%	120.00%	80.00%
100%	99.61%	2.84%	2.95%	8.69%	108.30%	90.93%	120.00%	80.00%
125%	100.65%	2.21%	2.21%	6.52%	107.16%	94.13%	120.00%	80.00%

Conc: concentration; RSDR: Relative Standard Deviation for Repeatability; RSDIP: Relative Standard Deviation for Intermediate Precision; CI: Confidence Interval; UB: Upper Bound; LB: Lower Bound; SL: Superior Limit, IL: Inferior Limit. $CI = RSDIP \times t$; $UB = Accuracy + CI$; $LB = Accuracy - CI$; $SL = 100 + \beta$; $IL = 100 - \beta$.

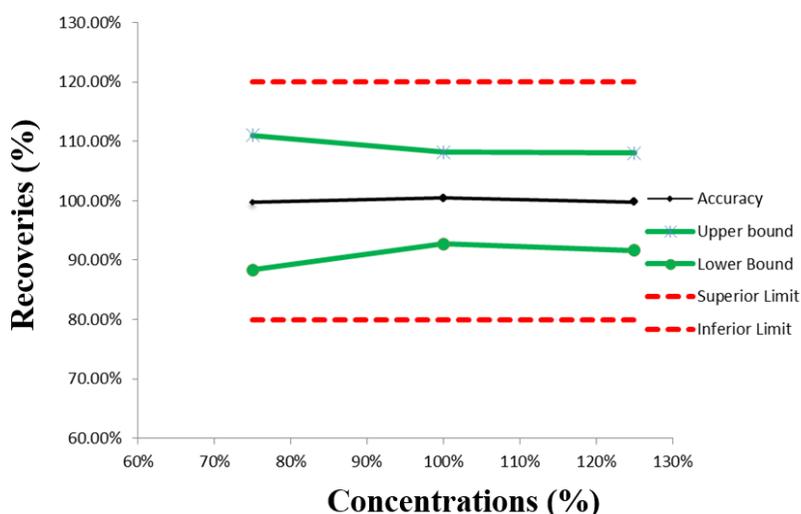


Figure 4: Method based on accuracy profile.

The method is validated as the accuracy profile is within the limits set for the assay.

DISCUSSION

UV-Visible spectroscopy is a simple, fast and efficient analysis technique for the quality control of drugs. The methods described in the American and British Pharmacopoeias, for the quality control of chlorpheniramine maleate, are based on a prior extraction of the compound from the pharmaceutical product. The methods are also tedious, requiring multiple steps and several solvents (ether, sulfuric acid, sodium hydroxyde, etc...) (MHRA 2009; USP 2013). The handling of such solvents requires most specific equipment. In addition, the extraction step presents a high risk of loss of active substances as well as the possibility of mistaking the phases to be recovered during handling. Finally, the methods described in the two pharmacopoeias require at least 2 hours to process a sample. On the contrary, the method presented in this study and validated, is a particularly low cost method with a low consumption of organic solvents (that are expensive and harmful to the environment) and easy quantification. It is run in 25 to 30 minutes for the analysis of a sample, requires no prior extraction of Chlorpheniramine maleate from the medicine, uses a simple equipment (UV-Visible spectrophotometry) and requires only 75 mL sulfuric acid for the analysis of a sample.

The validation of the method led to evaluate various criteria. From which the selectivity with a maximum absorbance of the spectrum at 265 nm is similar to the maximum absorbance of the United States Pharmacopeia (USP) method confirming the selectivity of the method. This selectivity is also confirmed by the method's linearity in the interval 0.018 to 0.3 mg/mL with a correlation value R^2 of 0.9993. This correlation was almost equal to the R^2 at 0.9998 obtained in a study which has validated a spectrophotometric method for the determination of the same compound in bulk and controlled released tablets (Ashfaq *et al.*, 2018).

The trueness was expressed in recoveries ranging from 95.13 to 104.34%. Those values are close to recoveries ranging

from 97.9 to 102.8%, found in a research where a Reverse Phase (RP) -High Performance Liquid Chromatography (HPLC) method has been validated for a simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation (Acheampong *et al.*, 2016).

The LOD and LOQ are respectively 1.39×10^{-4} and 2.26×10^{-3} mg /mL. Those values are high in comparison to a study which validate a fluorescence spectrophotometric method for the detection of chlorpheniramine maleate in pharmaceutical formulations with LOD and LOQ respectively at 0.03 and 0.09 $\mu\text{g/mL}$ (Azmi *et al.*, 2017). This difference could be explained by the fact that fluorescence spectrophotometric has been demonstrated to be more sensitive than UV-visible spectrophotometry. On the contrary, the values obtained in this study were very low in comparison to the ones obtained in a research designated for the validation of a spectrophotometric method for the same compound in bulk and controlled released tablets (Ashfaq *et al.*, 2018). Indeed, in this work, 2.2 and 6.6 $\mu\text{g/mL}$ were found for LOD and LOQ. This difference proves the present method to be more sensible as an UV-visible spectrophotometric method but also simple because the afore-mentioned study was requiring a prior extraction.

An acceptance limit β at 20%, is far from current standards that set a limit of 5% to 10% for medicines. Indeed, in the USP, 10% is the limit and 2% in the British Pharmacopeia (BP). This value was higher than the one in our previous research on the use of the same method to evaluate the quality of Efavirenz in tablets (Tittikpina *et al.*, 2020). But, acceptance limit set to this high value can be accepted for methods which allow the detection at very low concentrations like in this work (0.018 to 0.03 mg/mL) as documented in various researches (Zenzen *et al.*, 2012; Mottier *et al.*, 2016). Nevertheless, this method is offering beneficial conditions in comparison to other techniques with

regards to, length, price, easiness, sensitivity and accuracy.

Conclusion

The quality control of medicines remains an essential practice in public health. For developing countries this control is not always easy as compendial methods are expensive, lengthy and require difficult-to-handle equipment with various consumables not always available in developing countries. Although, any method chosen for routine analysis should be reasonably simple, the materials used should be readily available in the laboratory or readily accessible. This study consisted in validating a method of analysis without extraction applicable to a molecule in order to detect under-dosages, over-dosages as well as impurities. The study focused on chlorphenamine maleate, an essential drug used against allergies. The parameters necessary for validation were evaluated and the accuracy profile obtained was satisfactory. The validated method using UV-VISIBLE spectrophotometry is simple, fast, accurate, inexpensive, and can be applied routinely for quality control of tablets made of chlorpheniramine maleate. This method derives its importance from the term "without extraction" which, in addition to the advantages mentioned above, also limits the losses generated by extractive methods which are long and often poorly reproducible, requiring organic solvents which are difficult to handle. The UV-Visible spectrophotometer could rightly occupy a privileged position in the instrumental analytical device for controlling the quality of drugs, particularly in countries with limited resources.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

LCDC performed the different experiments. SMN helped with the running of the experiments with the UV-VIS spectrophotometry. NKT helped analyze the

data and wrote the manuscript. SOS designed the research. SOS, YMD, DF, BN and AD supervised the research. All the authors read, improved the paper and accepted its final version.

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