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Influence of Some Physico-Chemical Exposure Factors on the Carbocysteine Content of an Opened Pharmaceutical Product

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Abstract

The aim of this study was to investigate the stability of Carbocysteine (CBC) contained in a reference substance (RS) and in a sample of opened RHINATHIOL® 5% syrup (RNTL 5%) under the effect of some physico-chemical parameters (temperature, light, time, pH, bile salts). The developed method was linear, precise and accurate according to USP 38. The coefficient of determination R^2 for linearity was 0.9993. The respective RSD of intra-day and inter-day between days (1st, 2nd and 3rd day) were respectively 0.338% and the interval from 0.05% to 0.387%. The average recovery rate ranged from 98.490% to 100.450%. The detection and quantification limits were 0.0001 mg/mL and 0.001 mg/mL respectively. The method was applied to four samples of opened syrup containing CBC and the CBC content in these samples was found to be in accordance with USP 38. The CBC content in the opened sample of RNTL 5% was obtained by UV-visible spectrophotometry at 217 nm and was 4.887 g/100mL. The study of the influence of physico-chemical factors on the content of CBC in RS and RNTL 5% showed that the evolution of CBC contents in each drug matrix remained dependent on pH and temperature. However, these levels remained stable in the presence of light.

Keywords

Influence, Physico-Chemistry, Content, Carbocysteine, Stability

1. Introduction

In developing countries, the problem of the stability of pharmaceutical products

can have serious consequences on the health of the population such as the development of resistance to the usual treatment, and the deterioration of the state of health of the patient which can lead to death and the risk of fatal poisoning [1]. These consequences are caused by a decrease in the content of the active ingredient (API) contained in the drug, and the formation of numerous degradation products of preservatives and excipients [2].

In order to prevent these long-term problems, manufacturers have set up stability tests to determine the behaviour of the AP in the drug under different storage conditions in order to improve its stability [3]. These tests are recommended by the Q1A-Q1F standards established by the International Council for Harmonisation (ICH) of Technical Requirements for the Registration of Medicinal Products for Human Use [4]. However, the duration of validity of medicinal products attributed following these stability studies is not an infallible guarantee and remains dependent on the conditions of storage, transport, reconstitution or use [5] [6] [7]. Jassim A-M [8] and Shankar P. *et al.* [9] have illustrated in their various studies that medicines are stored inappropriately in households. This is the case in Côte d'Ivoire where some patients keep their opened medicines in different storage conditions beyond the validity of the treatment [10].

In order to address the different conditions that can impact the stability of the active ingredient contained in a drug, the choice of CBC chemically called (2R)-2-amino-3-[(carboxy-methyl) sulphanyl] propanoic acid [11] was made for the realization of this study because it is very solicited in Côte d'Ivoire in the treatment of recent respiratory affections with difficulty in spitting (difficulty in rejecting bronchial secretions by spitting) [12]. It is a molecule that appears as a white, crystalline powder, insoluble in water and alcohol. It dissolves in dilute solutions of mineral acids and alkali hydroxides [11]. It is derived from a thiolated amino acid (**Figure 1**) and its toxicity has only been evaluated in animals [13].

Several analytical techniques can be used to perform this study. These include High-Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), UV-visible Spectrophotometry [14], Fluorimetry [15] and Titrimetry [16] [17] [18]. UV-visible spectrophotometry was chosen for the implementation of this study because it is a popular analytical technique that allows the quality of a sample to be checked quickly and reliably [19].

The objective of this study was firstly to set up an assay method to quantify CBC in tapped samples and secondly to study the impact of some physico-chemical exposure factors on the CBC content of one of the tapped samples (the RNTL 5%).

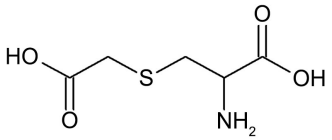

Figure 1. Chemical structure of carbocysteine (CBC).

2. Materials and Method

2.1. Sampling

Four opened syrups containing CBC 5% with a shelf life of one year after use were used for this study (Table 1). RNTL 5% was used in the following for the study of influencing factors.

2.2. Reference Substance and Chemicals

Carbocysteine (CBC) of 99% purity and batch number 5MJ175 supplied by the manufacturer GPHF (Global Pharma Heath Fund) was used as the reference substance (RS). Purified water and 0.1N NaOH (dilution solution) were respectively used throughout the study to prepare the different solutions. Solutions such as: 0.1N NaOH, 0.1N HCl and 0.54 mg/mL bile salt solution were used to carry out the study. These different solutions were obtained from reagents such as NaOH of 97% purity, HCl of 37% purity and bile salts of 100% purity. They were supplied by the manufacturers CARLO ERBA and FLUCA analytical.

2.3. Apparatus

A double-beam UV-visible spectrophotometer SPECOR 210 PLUS from the manufacturer ANALYTIK JENA and a pH meter from HACH were used to measure the absorbance of the solutions obtained and to measure the pH of the different solutions, respectively.

2.4. Method

2.4.1. Preparation of the Stock Solution of CBC Reference Substance (RS)

The stock solution of CBC RS was prepared by dissolving 10 mg of CBC in 10 mL of 0.1 N NaOH to obtain a solution of concentration 1 mg/mL.

2.4.2. Preparation of CBC Daughter Solutions

From the initially prepared CBC stock solution, daughter solutions were prepared at concentrations of 0.02 mg/mL, 0.04 mg/mL, 0.05 mg/mL, 0.06 mg/mL, 0.08 mg/mL and 0.1 mg/mL.

2.4.3. Preparation of Sample Solutions

100 mg of sample syrup was weighed and then transferred to 100 mL volumetric flasks (four). All flasks were made up to the mark with 0.1 N NaOH to give concentrations of 1 mg/mL. 0.5 mL of each of the above solutions was then taken and placed in a further 10 mL volumetric flasks (four) to give a concentration of 0.05 mg/mL.

2.4.4. Development of the Assay Method

Validation parameters such as: linearity, precision, accuracy and limits of detection and quantification were evaluated according to the IHT validation procedures [20] to set up the assay.

- **Linearity**

Table 1. List of samples used for the study.

Pharmaceutical specialty	Dosage	Coding	Batch numbers
RHINATHIOL®	5%	RNTL	8K1401
FLUDIBRONC®	5%	FDBC	20119/2
MEDIBRONC®	5%	MDBC	V002
CARBOTOUX	5%	CBTX	19P0509A

It was evaluated by determining the coefficient of determination R^2 deduced by the calibration curve. It ranged from 0.02 to 0.1 mg/mL. A spectral scan in the range of 200 to 400 nm was performed to determine λmax. It was 217 nm. The spectrophotometer was then configured at 217 nm for the taking of readings of each daughter solution (n = 3). The average of the absorbances corresponding to each concentration level of the daughter solutions was used to plot the CBC RS calibration curve (Figure 2).

- **Loyalty**

Six separate 10 mL volumetric flasks containing daughter solutions of the RS standard at C = 0.05 mg/mL were prepared. Repeatability or intra-day precision was determined by taking three readings (n = 3) for each volumetric flask in the spectrophotometer on the same day (Table 2). The inter-day precision was determined by also taking three readings (n = 3) per volumetric flask on three consecutive days with the daughter solution of RS standard at C = 0.04 mg/mL. The average absorbance of the three sets of readings/vial and the coefficients of variation (RSD) were calculated for each test (Table 3).

- **Accuracy**

It was carried out by the metered addition method. To a quantity corresponding to 50 µg of the sample solution, three different additions of 40 µg, 50 µg and 60 µg of the reference solution RS were made to three separate 10 mL volumetric flasks.) The absorbances obtained after reading were converted to µg /mL using the equation line and then to µg (by multiplying the concentration obtained from the equation line by the total volume of the different mixtures). The recovery rate was then determined (Table 4).

- **Limit of detection (LOD) and limit of quantification (LOQ)**

These were assessed by successive dilutions of CBC RS at 0.02 mg/mL to 1/2, 1/10, 1/20 and 1/200 (Table 5).

2.4.5. Determination of CBC Content in Syrup Samples

The content of CBC in the 5% CBC syrup samples was quantified and the data are listed in (Table 6).

2.4.6. Influence of Physico-Chemical Parameters on CBC Content: Case of RNTL 5%

- **Influence of physical parameters**

The influence of temperature was studied by preparing three paired solutions of RS and RNTL 5% at 0.05 mg/mL and subjecting them to different tempera-

tures (25˚C, 30˚C, 37˚C and 40˚C) for one hour. The absorbance versus temperature curve was plotted (Figure 4). The previously prepared pairwise solutions were exposed to laboratory light for four successive days. The absorbances were measured each day after exposure to light and noted. The values obtained were used to plot the influence of light on the CBC content of RS and RNTL 5% as a function of time (Figure 5).

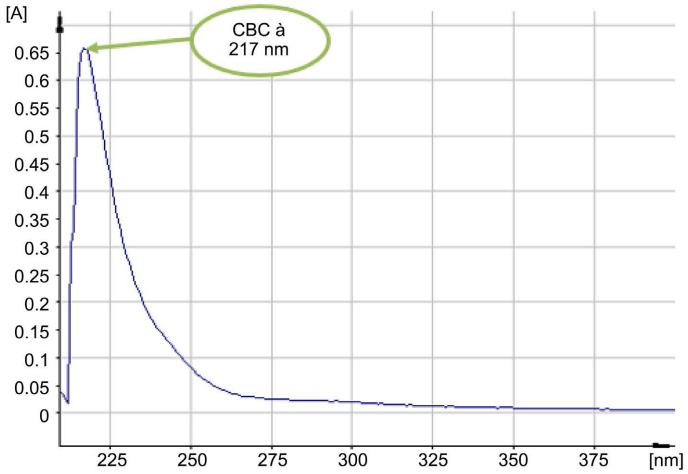

Figure 2. Absorption spectrum of a CBC daughter solution at 217 nm.

Table 2. Intra-day reliability of the method.

Concentration (mg/mL)	**Average absorbance**	**Mean ± Standard deviation**	**RSD** (%)
0.05	0.530		
0.05	0.533		
0.05	0.534	0.532 ± 0.002	0.338
0.05	0.530		
0.05	0.534		
0.05	0.532		

Table 3. Inter-day reliability of the method.

Concentration (mg/mL)	**Day 1**	**Day 2**	**Day 3**
0.04	0.382	0.435	0.261
0.04	0.381	0.438	0.263
0.04	0.383	0.436	0.262
0.04	0.381	0.437	0.263
0.04	0.379	0.439	0.262
0.04	0.377	0.436	0.262
Average	0.381	0.437	0.262
Standard deviation	0.002	0.002	0.001
RSD (%)	0.050	0.387	0.200

Table 4. Accuracy of the method.

Sample quantity (µg)	Amount of added RS (µg)	Total amount recovered (µg)	Recovery rate (%)
50	40	8.930	**98.90**
50	40	8.947	
50	50	9.915	**100.000**
50	50	10.085	
50	60	10.960	**100.450**
50	60	11.090	

Table 5. Limit of detection and limit of quantification of the method.

Dilution factor	Concentration (mg/mL)	Absorbances
1/2	0.0100	0.199
1/20	**0.0010**	0.020
1/200	**0.0001**	0.000

Table 6. Amount of CBC contained in the samples.

Samples	Average absorbance	Quantity CBC (g/100mL)	USP Standard 38 (g/100mL)
RNTL 5%	0.505	4.887	
FDBC 5%	0.483	4.740	4.500 à 5.500
MDBC 5%	0.500	4.964	
CBTX 5%	0.506	5.030	

- **Influence of chemical parameters**

Eleven paired solutions of RS and RNTL 5% at 0.05 mg/mL were prepared and placed in contact with 0.1 N HCl for one hour in order to obtain solutions with a pH of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 respectively. The reading of these different solutions made it possible to obtain the evolution curve of the pH and the concentration of CBC contained in the RS and the RNTL 5% (**Figure 6**). These same two solutions (one RS and one CBC) were also put in contact with 10 mL of 0.54 mg/mL bile salt solution. A contact time of one hour was also observed before the different readings were taken.

2.5. Expression of Data

The data were obtained using MICROSOFT EXCEL 2016 software and were expressed as mean ± standard deviation and RSD (%). They were also compared to the USP 38 pharmacopoeia data [21].

3. Results and Discussion

- **Determination of the detection wavelength λmax**

Prior to the study of the validation parameters, we proceeded to the determination of the maximum absorption wavelength λmax of the CBC by performing a spectral scan from 200 nm to 400 nm. This spectral scan allowed us to detect the maximum absorption wavelength λmax of the CBC which is 217 nm. This λmax is substantially identical to that obtained by Rele R *et al.* [22] which was 215 nm. On the other hand, this λmax differs from that performed by Pargaonka G *et al.* [18] who found a λmax of 259 nm. This difference in wavelength could be justified by the fact that in the present study, the CBC was dissolved in an aqueous medium and the absorbance reading was done directly with the spectrophotometer whereas in the study of Pargaonka G *et al.* [18] a complexation reaction between the CBC and nickel in a non-aqueous medium was required prior to the spectrophotometer reading.

- **Linearity**

This was determined by assessing the coefficient of determination R^2 obtained from the calibration curve. This coefficient was 0.9993. Our result is in accordance with USP 38 [21] which indicates that the coefficient of determination must be greater than 0.9950. Our method is therefore linear (**Figure 3**). Our result is superposable to the R^2 obtained by Chauhan K *et al.* [23] in their study on the validation of a liquid chromatography (HPTLC) method for the determination of CBC in pharmaceutical products.

- **Loyalty**

It was evaluated by intra-day and inter-day fidelity, which each gave respective RSD of 0.338% for the former (**Table 2**) and 0.050% to 0.387% for the latter over three successive days (**Table 3**). Our method complies with the RSD given by USP 38 [21] which stipulates that the RSD for intra-day and inter-day fidelity should be less than 1% and 1.5% respectively. Therefore our method is faithful.

- **Accuracy**

It gave average recovery rates that ranged from 98.490% to 100.450% for CBC RS quantity levels of 40 µg, 50 µg and 60 µg added to the sample solution. These values are in accordance with the specifications given by USP 38 Pharmacopoeia [21] which recommends that the average recovery rate be contained within the acceptability range of 98% to 102%. Our method is therefore accurate like Rele Rajan V [24] who found an average recovery rate of 100.74% in his study (**Table 4**).

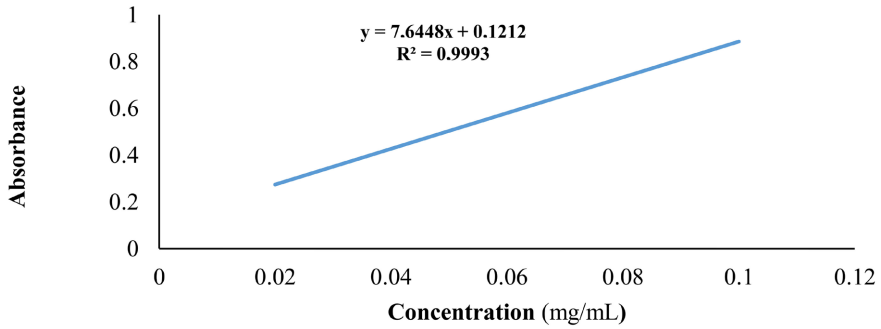

Figure 3. CBC RS calibration curve.

- **The limit of detection (LOD) and the limit of quantification (LOQ)**

These are 0.0001 mg/mL for the limit of detection and 0.0010 mg/mL for the limit of quantification. These different limits highlight the sensitivity of our method for the determination of CBC in RNTL 5% syrup (Table 5). These limits of detection and quantification are superimposed on those found by Rele Rajan V [24] which were 0.0004 mg/mL and 0.0012 mg/mL respectively.

- **Quantification of CBC in syrup samples**

The content of CBC in our syrup samples was 97.73% or 4.887 g/100mL for RNTL 5%, 94.80% or 4.740 g/100mL for FDBC 5%, 99.28% or 4.964 g/100mL for MDBC 5% and 100.59% or 5.030 g/100mL for CBTX 5%. These results are in accordance with the specifications given by the USP 38 Pharmacopoeia [8] for the assay of samples, which stipulates that the content of active ingredient must be between 90% and 110% of the quantity mentioned on the secondary packaging of the drug.

- **Influence of physico-chemical factors: the case of RNTL 5%.**

When the temperature varies from 25˚C to 40˚C, the CBC concentrations in the RS (reference) and in the tapped syrup of RNTL 5% (sample) evolve respectively from 0.0201 mg/mL to 0.057 mg/mL for the former and 0.047 mg/mL to 0.089 mg/mL for the latter. This evolution could be explained by the fact that by-products of this rise in temperature appear, and these would absorb at λmax = 217 nm. The results obtained differ from those of Taha E.A *et al.* [25], who obtained a decrease in absorbances as the temperature increased. These results show that temperature has an influence on CBC by changing its content (Figure 4). Regarding the effect of light, the lack of variation in CBC concentration in RS and RNTL 5% could suggest that light does not influence CBC content after the four days tested (Figure 5). For pH values ranging from 12 to 7, the concentrations of CBC in RS and RNTL 5% decrease rapidly from 0.031 mg/mL to 0.0007 mg/mL for the former and from 0.044 mg/mL to 0.0055 mg/mL for the latter. They stabilise at pH values below 7 (Figure 6). This result could be explained by the fact that after pH = 7, all the quantities of CBC contained in these drug matrices have been degraded or neutralised, so there is no more CBC in these solutions. The pH therefore has an influence on the CBC content of these drug matrices (CBC and RNTL 5%). The contact of RS and RNTL 5% with the bile salt solution showed a slight increase in the CBC concentration. From 0.06 to 0.08 mg/mL in RS and from 0.017 to 0.0206 mg/mL in RNTL 5% (Table 7). This would indicate the appearance of secondary products that would absorb in this

Table 7. Evolution of the amount of CBC under the influence of bile salts.

	Concentration CBC (mg/mL)	
Time (min)	**RS**	**RNTL 5%**
0	0,006	0.017
60	0,008	0.0206

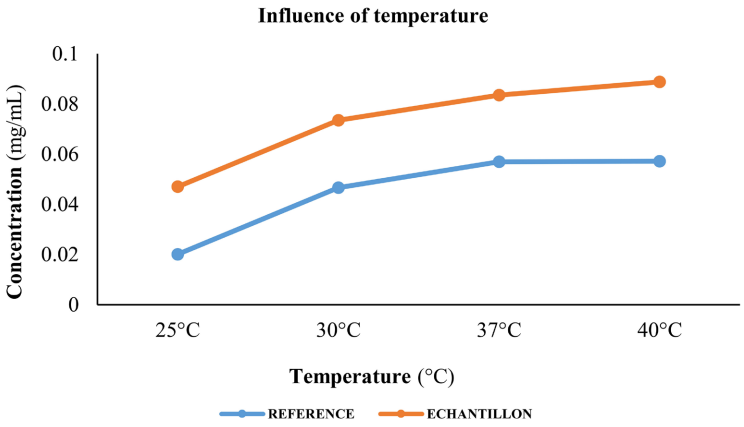

Figure 4. Evolution curve of the impact of temperature variation on CBC contents.

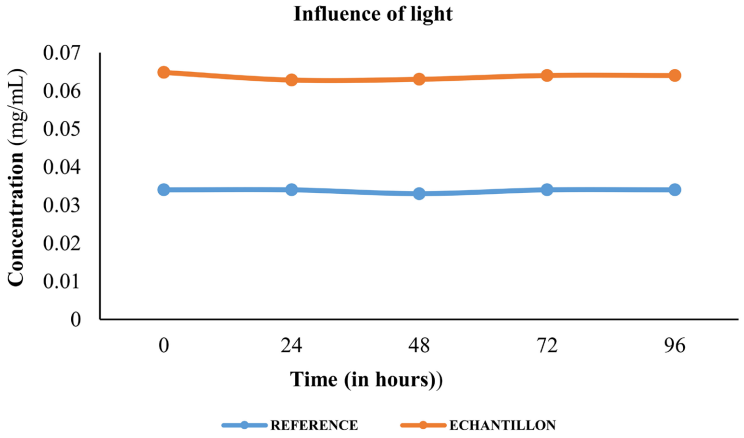

Figure 5. Evolution curve of the impact of light over time on CBC levels.

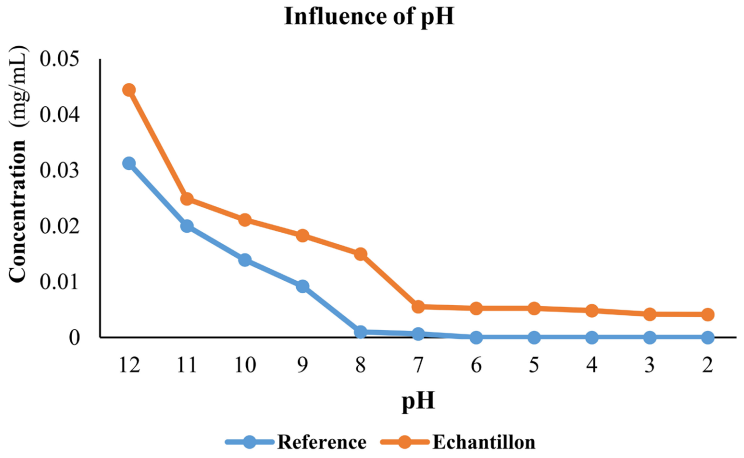

Figure 6. Evolution curve of the pH variation on the CBC contents.

same wavelength range (200 nm to 400 nm). Bile salts therefore have an influence on CBC. It would therefore be wise to administer CBC away from meals to reduce the impact of bile salts, which are produced in abundance in the digestive

tract during large, fatty meals. This result is in line with the manufacturers' recommendations that CBC should be taken away from meals [12].

4. Conclusions

The method for the determination of CBC in the opened syrup samples developed according to IHT guidelines during the study was satisfactory, *i.e.* linear, precise and accurate. It was therefore possible to determine the quantities of CBC contained in these opened syrup samples. These analysed quantities were thus in conformity with the specifications of the USP 38 pharmacopoeia version 2015.

Subsequently, RS and RNTL 5% were exposed to different physico-chemical parameters (temperature, light, bile salts and pH) to study the behaviour of CBC in these different matrices. It was found that the variation of the amount of CBC in the matrices was proportional to the variation of pH and temperature and stable over time in the presence of light. As the influence of light on the CBC content was limited to four days, further tests will have to be carried out in order to determine the fate of the influence of light on the CBC content of these drug matrices in the long term. The evolution of the CBC content in the opened syrup of RNTL 5% is almost identical to that in the RS when the physico-chemical conditions under which these drug matrices are found are varied.

Nevertheless, in order to highlight the consequences of improper storage of opened medicines and to better understand the behaviour of several active ingredients with respect to these numerous exposure factors, it would be more judicious to extend this study to the other pharmaceutical specialities identified in this study with a wide range of in vitro physicochemical exposure factors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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