ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2021, *66*(4), 367–373
DOI: 10.33224/rrch.2021.66.4.08

EXTRACTIVE SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF CYPROHEPTADINE HYDROCHLORIDE USING ALIZARIN RED S

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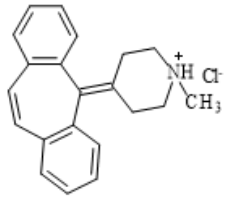
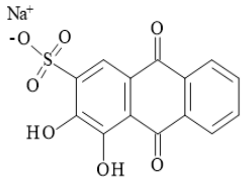
Received December 11, 2020

Three new spectrophotometric methods, based on ion pair formation, have been developed for the assay of cyproheptadine hydrochloride from bulk and tablets. In method A, the yellow ion-pair, cyproheptadine – alizarin red S, obtained in acidic medium, was quantitatively extracted into chloroform and the absorbance of the chloroformic solution was measured at 425 nm. In the methods B and C, the ion-pair was broken in alkaline medium and the absorbances of the alcohol-chloroformic solution were measured (at 524 nm for method B and at 560 nm for method C). Good linear relationships between absorbance and the concentration of cyproheptadine hydrochloride in the ranges of 15–45 µg / mL (method A), 9–43 µg / mL (method B) and 2.5–18 µg / mL (method C) were found. The molar ratio, the values of molar absorptivity, limits of detection and quantification were determined.



Cyproheptadine hydrochloride



Alizarin Red S

INTRODUCTION

Cyproheptadine hydrochloride (CYPHCl), 4-(5H-dibenzo [a,d] cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate, has antihistaminic and anti-serotonin properties.[1] It acts in the gastrointestinal tract, large blood vessels and bronchial smooth muscles. CYPHCl reveals anticholinergic and sedative properties and stimulates appetite and weight gain, also. It is prescribed for allergic manifestations.[2] In a recent clinical trial, the authors found a similar efficiency of cyproheptadine and amitriptyline as suggested medications for prophylaxis of cyclic vomiting syndrome.[3] According to some data from the literature, it seems that cyproheptadine can be useful in the treatment of neuropsychiatric disorders that are related to retroviral and/or HIV infection.[4] As a potent 5-HT2A receptor antagonist and a well-tolerated drug compared to typical antipsychotic drugs, cyproheptadine would qualify as a candidate for prophylactic treatment of fatal disorder typically occurring in immunosuppressed patients as a consequence of HIV infection, as well as treatment with various biological response modifiers.[5] Also, the treatment of human breast cancer cells with cyproheptadine suggest that this drug substance can be reconsidered for breast cancer treatment or used as a starting point for the discovery of an anti-hormone breast cancer.[6]

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Given the widespread use of cyproheptadine to prevent or treat various symptoms, simple and inexpensive assay methods are required. The drug is official in European Pharmacopoeia 10th Edition that reports a potentiometric method for the assay of CYPHCl.[1] Until now, many analytical methods including titrimetry,[7,8] electrometry,[9,10] spectophotometry[11-15] and chromatography[16-20] have been developed for the assay of CYPHCl in bulk, biological samples and pharmaceutical formulations.

Taking into account that the spectrophotometric methods are considered convenient techniques for drugs assay, especially in pharmaceutical dosage forms, we have directed our research in this direction. There are many spectrophotometric methods reported in literature for determination of drugs in bulk or pharmaceutical dosage forms.[21-26] Some of these are extractive spectrophotometric methods based on the property of drugs to form ion-pair complexes with different reagents.[27-29] Alizarin red sulfonate, 3,4-dihydroxy-9,10-dioxo-2-antracenesulfonic acid sodium salt (ARS) has been used as a reagent in the spectrophotometric determination of some drug substances.[30-31] The reaction between ARS and CYPHCl has not been investigated yet. It is clearly visible from the structure of cyproheptadine which contains a nitrogen atom that are theoretical premises for the formation of ion pair between CYPHCl and ARS, so we studied this reaction. This paper describes three new spectrophotometric methods and their experimental conditions for the assay of CYPHCl in bulk and pharmaceutical formulations.

RESULTS AND DISCUSSION

Three UV-VIS spectrophotometric methods for determination of CYPHCl are described. Separation of studied drug from aqueous solution is based on the capacity of chloroform to extract the ion pair formed between CYPHCl and ARS.

There are three acidic groups in the structure of ARS. The effect of different organic-water mixtures on the acidity constants and ionization of phenolic groups of ARS was determined by ~~a~~ spectrophotometric methods.[32-33] At pH < 5, the aqueous solution of ARS is yellow and turns violet at pH > 11, depending on ionization of phenolic groups (weak acidic, pKa1 = 5.5, pKa2 = 11) and exhibits characteristic absorbance in the visible range. In strong acidic medium, the two phenolic functions are non-ionized and spectrum of ARS exhibits maximum of absorbance at 425 nm. At pH values between 9.0 and 11 the colour turns from red to violet. At pH < 10 only one phenol function is ionized and two absorption peaks are observed at 326 and 520 nm. In basic medium two peaks can be observed, at 550-556 and 585–596 nm.[34-35]

In acidic medium, the cation $CYPH^+$ forms with the anionic form of ARS (ARS^-) a yellow $CYPH^+ \cdot ARS^-$ ion pair extractable into chloroform. The absorbance of the yellow chloroformic solution was used for CYPHCl assay by proposed method A.

The $CYPH^+ARS^-$ ion pair formed in acidic medium and extracted in chlorofom, was unstable at the addition of KOH. Because ethanol and methanol are miscible with chloroform, upon the addition of alcoholic KOH solutions to the chloroformic solution of $CYPH^+ARS^-$ ion-pairs, the weakly acidic groups of alizarin red S (-OH groups) underwent progressive ionization. The $CYPH^+ \cdot ARS^-$ ion pair was broken and free ARS anions colored the solution in red–violet or violet. Measuring the absorbance of red-violet or violet chloroformic solutions was used for spectrophotometric determination of CYPHCl by method B and method C.

Aqueous solution of CYPHCl is colorless and it has the UV absorption maximum at 286 nm in acidic solution and at 284 nm in alkaline solution.[3] In the proposed methods, bathochromic shift of the UV absorption maximum, allowed interference-free procedures establishing for the assay of cyproheptadine.

Optimization of experimental conditions

Extraction solvent selection. In order to choose the most appropriate solvent for the quantitative extraction of ion pair from aqueous solution, several organic solvents (carbon tetrachloride, dichloromethane and chloroform) were tested. We've considered polarity of the solvents and of the ion-pairs (with polar groups in ARS^- anion structure) and volatility of the solvents. Chloroform, with medium polarity and a boiling point of 61.2°C (lower than carbon tetrachloride, higher than dichloromethane) was chosen to selectively extract the ionic association from the aqueous phase. The dielectric constant of chloroform, prevents $CYPH^+ \cdot ARS^-$ ion pair dissociation into free ions and ensures its extraction in the organic solvent.

Measurement wavelengths selection. The absorption spectrum of the chloroformic solution of $CYPH^+ \cdot ARS^-$ ion pair shows an absorption maximum at λ = 425 nm (Fig. 1 a). The ethanolic - chloroformic solution shows maximum of absorbance at λ = 524 nm (Fig. 1 b) and the maximum of absorption of methanolic – chloroformic solution was recorded at λ = 560 nm (Fig. 1 c). These wavelengths, 425 nm, 524 nm and 560 nm were used in subsequent measurements, in the proposed methods A, B and C, respectively.

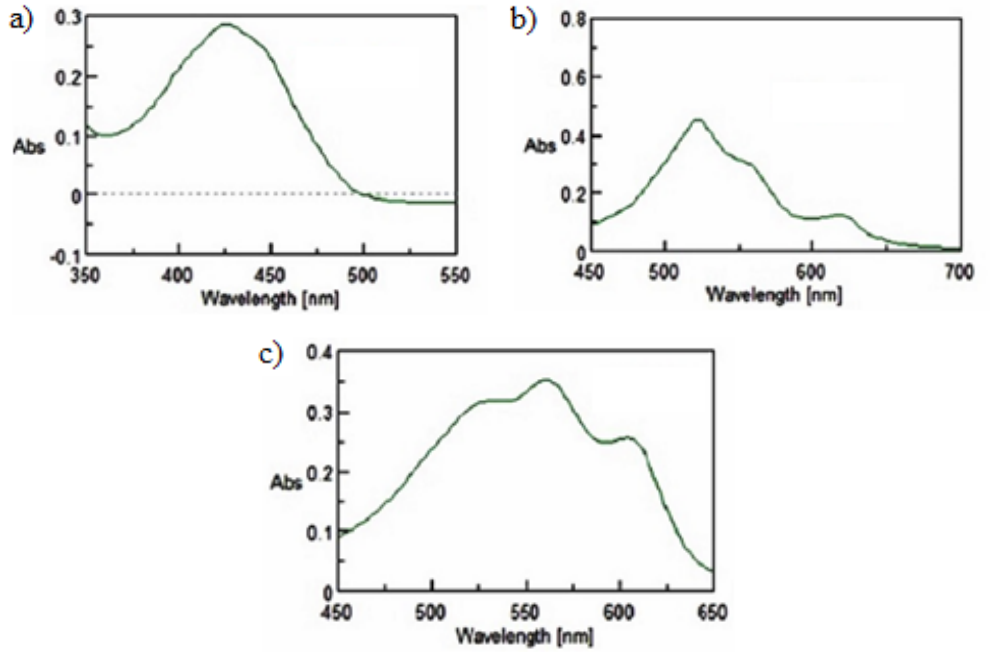

Fig. 1 – Absorption spectrum recorded: a) method A (22 μg CYPHCl / mL), b) method B (21 μg CYPHCl / mL) and c) method C (15 μg CYPHCl / mL).

Effect of time on the stability of the chromophore groups. The effect of time was studied by measuring the absorbance at 425 nm, 524 nm and 560 nm (in chloroformic, ethanol-chloroformic and methanol-chloroformic solutions, respectively), over 1 hour, at 10 minutes intervals. The absorbance is constant for at least 50 minutes for the chloroformic and methanol-chloroformic solutions, sufficient time for the analysis of a large number of samples. The absorbance profile for the ethanol-chloroformic solution of the ionic association shows a decrease of the absorbance in the first 20 minutes, then the absorbance is constant for 30 minutes. In this case we chose to record the absorption spectra 25 minutes after the KOH solution addition.

Effect of ARS volume. ARS concentration in aqueous phase determines the colour intensity of chloroformic solutions. This effect was studied by measuring the absorbance of the organic phase at 425 nm, after $CYPH^+ \cdot ARS^-$ ion pair extraction from 20 mL acidic aqueous solutions containing same concentration of CYPHCl and different amounts of ARS. The maximum colour intensity and maximum value of absorbance was achieved with 6 mL ARS 0.05% solution providing an excess of dye (Fig. 2).

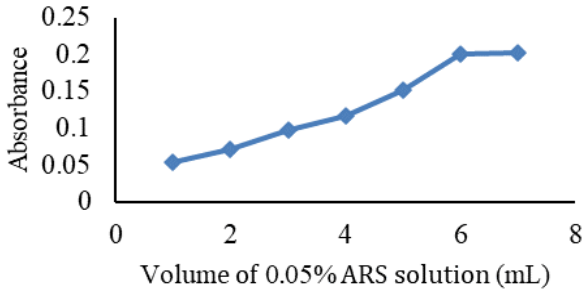

Fig. 2 – Effect of ARS volume on the absorbance value of $CYPH^+ARS^-$ ion-pair (17 μg CYPHCl/mL).

Effect of shaking time. It was measured the value of absorbance of the organic phase after $CYPH^+ARS^-$ ion pair extraction from 20 mL acidic aqueous solutions containing fixed amounts of CYPHCl and ARS after different shaking times with 10 mL of chloroform. The results obtained reveal that the maximum value of absorbance was reached after 2 minutes of shaking (Fig. 3).

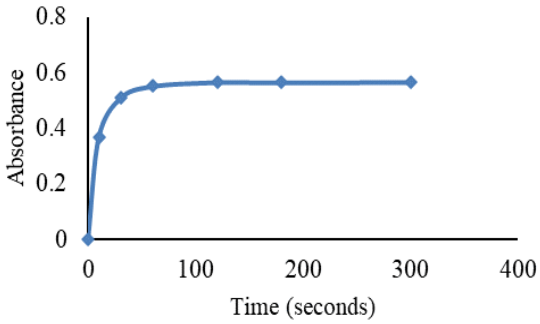

Fig. 3 – Effect of shaking time (45 µg CYPHCl / mL).

Stoichiometry of ion pair complex was established by Job`s method of continuous variations of equimolar solutions.[37] Three standard solutions of CYPHCl ($3.5 \cdot 10^{-3}$ M; $4 \cdot 10^{-3}$ M and $4.5 \cdot 10^{-3}$ M) and three standard solutions of ARS ($3.5 \cdot 10^{-3}$ M; $4 \cdot 10^{-3}$ M and $4.5 \cdot 10^{-3}$ M) were prepared and then, 10 mL of each standard solution was diluted to 50 mL to obtain three working solutions for CYPHCl ($7 \cdot 10^{-4}$ M; $8 \cdot 10^{-4}$ M and $9 \cdot 10^{-4}$ M), respectively for ARS ($7 \cdot 10^{-4}$ M; $8 \cdot 10^{-4}$ M and $9 \cdot 10^{-4}$ M).

Three series of solutions in which the total volume of CYPHCl and ARS was kept at 10 mL were prepared: to 1.0 – 9.0 mL the working solutions of CYPHCl were added 9.0 -1.0 mL working solutions of ARS. After extraction in chloroform, the absorbance was measured at 425 nm against reagent blank. The maximum value for the absorbance was found for a molar ratio of 0.5. A 1:1 ($CYPH^+$: ARS^-) ion pair was formed through the electrostatic attraction between the positive ion $CYPH^+$ and negative ion ARS^- (Fig. 4).

The proposed reaction for the formation of $CYPH^+ \cdot ARS^-$ ion pair is presented in Fig. 5.

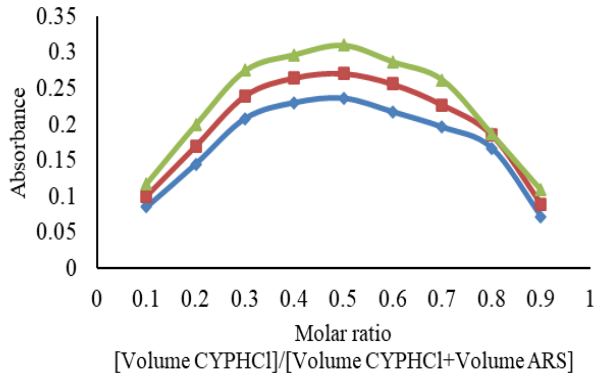

Fig. 4 – Job's continuous-variations plots of $CYPH^+ \cdot ARS^-$ ion pair.

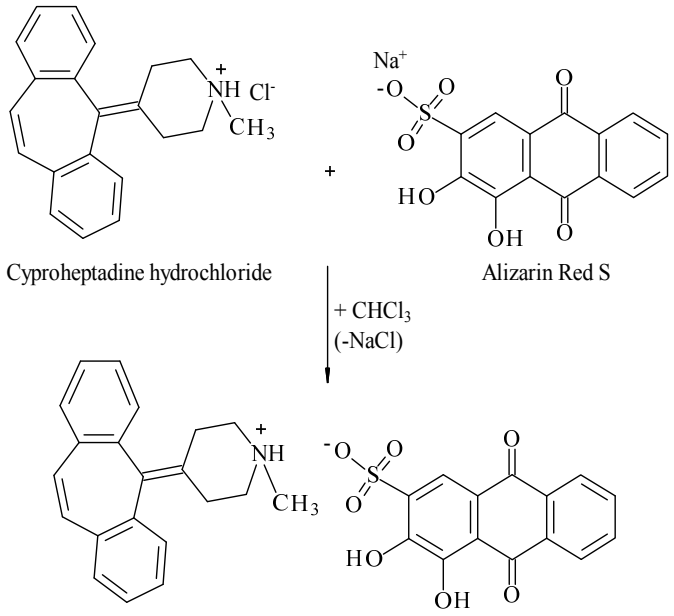

Fig. 5 – The proposed reaction for $CYPH^+ARS^-$ ion-pair formation (method A).

Analytical performances testing

The methods were validated according to the ICH regulations Q2 (R1).[38]

Linearity. Standard solutions of different concentrations of CYPHCl were prepared using procedures described at chapter *Experimental*. Standard calibration curves were constructed by plotting value of absorbance (at 425 nm for method A, at 524 nm for method B and at 560 nm for method C) versus concentration of CYPHCl. Linear correlations between absorbance and concentration of CYPHCl were found and were described by the regression equations with coherent correlation coefficients (Table 1).

Sensitivity. Detection limit (DL) and quantification limit (QL) were estimated (Table 1) using the standard deviation for the analysis of a blank reagent and the slope of the calibration curve.[39]

Accuracy of these three methods was evaluated. Samples of known concentrations of CYPHCl (concentrations of 80%, 100% and 120% of the value of interest) were assayed (Table 1).

Precision was determined both as repeatability and intermediate precision. Repeatability was determined using three samples of known concentrations; for each of these samples, three successive determinations were performed. The intermediate precision was studied by determinations on three different days with freshly prepared solutions (Table 1).

Assay parameters are similar to those obtained for the determination of CYPHCl by other spectrophotometric methods (Table 2).

Selectivity was evaluated by applying the methods for determination of CYPHCl from a synthetic mixture containing CYPHCl and adjuvants from *Peritol* tablets (4 mg CYPHCl anhydrous, 100 mg magnesium stearate and 90 mg potato starch). No interference was observed from these excipients in pharmaceutical dosage forms of CYPHCl, indicating high selectivity. For drug substance percent recoveries were 99.70±1.05 (method A), 99.85±1.55 (method B), 100.45±1.86 (method C).

Analysis of the pharmaceutical dosage forms

The proposed methods were successfully applied to the determination of CYPHCl in pharmaceutical dosage forms. Mean recovery for CYPHCl from Peritol tablets and confidence interval were calculated and results showed good agreement with the label claim (Table 3).

Table 1

Parameters of validation for determination of CYPHCl by the proposed methods

Analytical parameter	Method A	Method B	Method C
Measuring wavelength (nm)	425	524	560
Linearity range (μg/mL) *	15 – 45	9 – 43	2.5 – 18
Molar absorptivity, ε ($L \cdot mol^{-1} \cdot cm^{-1}$)	4046	7360	7231
$A^{1\%}_{1cm}$	125	227	223
Regression equation (y)	0.0139 x -0.037	0.0262x-0.0591	0.0204x +0.0179
Correlation coefficient (r)	0.9957	0.9919	0.9880
DL (μg/mL)	0.60	0.55	0.34
QL (μg/mL)	1.82	1.65	1.02
Accuracy (Confidence interval for 95% level of confidence)	99.88±0.60	100.29±0.67	100.35±0.82
Repeatability (R.S.D. %)	0.95	1.05	1.35
Intermediate precision (R.S.D. %)	0.91	1.23	1.57

* N = 7 (methods A and C), N=9 (method B), n = 3

Table 2

An overview on the (performance parameters of the) proposed methods compared to similar methods in the literature

Reagent	λ nm	Linear range µg/ mL	DL µg/ mL	QL µg /mL	RSD %	Reference
Alizarin red S	425	15 – 45	0.60	1.82	0.95	Proposed method A
Alizarin red S	524	9 – 43	0.55	1.65	1.23	Proposed method B
Alizarin red S	560	2.5 – 18	0.34	1.02	1.57	Proposed method C
Bromocresol purple	405	1.5–25	1.12	1.7	0.940	13
Chloranilic acid	520	25-125	1.07	3.57	1.96	11

Table 3

Results for determination of CYPHCL in tablets

Data	Method A	Method B	Method C
Mean recovery (%)	99.56	99.14	100.24
Confidence interval (%) *	99.56±1.25	99.14±1.27	100.24±1.55

* n = 3, N = 6

EXPERIMENTAL

Materials and Instruments

Jasco V- 730 spectrometer (absorption spectra were recorded in quartz cells, 1 cm path length, at 25±1ºC); analytical balance Mettler Toledo AT 261 (0.01 mg sensitivity); ultrasonic bath Elma 9331-1.

CYPHCl, cyproheptadine hydrochloride sesquihydrate 99% (Sigma Aldrich), alizarin red S (Fluka AG), potassium hydroxide (Merck KGaA), hydrochloric acid (Merck KGaA), ethanol (Merck KGaA), methanol (Merck KGaA) were used to obtain following solutions: 0.05% (w/v) alizarin red S aqueous solution, 0.2% (w/v) potassium hydroxide ethanolic solution, 1% (w/v) potassium hydroxide methanolic solution, 2 M hydrochloric acid aqueous solution. Sodium sulfate anhydrous (Merck KGaA), potato starch (Fluka) and magnesium stearate (Magnesia), chloroform for spectroscopy (Sigma Aldrich) were used as received. All the above materials were of analytical grade. Water was always distilled.

Stock solution was $1.7 \cdot 10^{-4}$M CYPHCl (0.06 g CYPHCl in 100 mL water). Working solution $3.4 \cdot 10^{-5}$ M was obtained by dilution with water.

Peritol tablets (Egis Pharmaceutical PLS, Budapest) purchased from local market was examined. This pharmaceutical dosage form was labeled to contain 4 mg of CYPHCl anhydrous / tablet and excipients: lactose monohydrate, magnesium stearate, gelatin, talc and potato starch.

General procedures

Procedure for drug determination

Method A: Volumes of 3.0–-9.0 mL of $3.4 \cdot 10^{-5}$M CYPHCl solution were transferred into a series of separating funnels. To each funnel, 5.0 mL of HCl 2 M and 6.0 mL of 0.05% ARS were added; the total volume was adjusted to 20 mL with water. The content of separating funnel was shaked well and the formed ion-pair was extracted into chloroform by shaking for 2 minutes with 10 mL chloroform once, and then, three times with 5 mL chloroform. The chloroformic layer was dried over anhydrous Na_2SO_4 and collected into a 25 mL volumetric flask. The volume was adjusted to 25.0 mL with chloroform. The absorbance of the yellow chloroformic solution was measured at 425 nm against blank sample.

Method B: Different aliquots of 1.5–3.5 mL of a chloroformic solution of ion-pair complex (corresponding to $6.162 \cdot 10^{-5}$ g CYPHCl /mL) prepared by procedure described for method A, were transferred into a series of 10 mL volumetric flask and the total volume was adjusted to 9.5 mL by adding chloroform. Then, to each flask, 0.5 mL of 0.2% ethanolic KOH was added. The content was mixed and left to stand for 25 minutes. The absorbance of red-violet chloroformic solution was measured at 524 nm against blank sample.

Method C: Different aliquots 0.5–3.5 mL of chloroformic solution of ion-pair complex (corresponding to $1.027 \cdot 10^{-4}$ g CYPHCl /mL) prepared by procedure described for method A, were transferred into a series of 10 mL volumetric flask and the total volume was brought to 8 mL by adding an adequate quantity of chloroform. Then, to each flask, 2 mL of 1 % methanolic KOH was added. The content was mixed and the absorbance of violet chloroformic solution was measured at 560 nm against blank sample.

Procedure for commercial tablets

The average tablet mass was determined by weighing 20 tablets (0.20027 g). Then, those 20 tablets were mixed and finely powdered. An amount of 2.5 g of powder was

accurately weighed and transferred to 100 mL volumetric flask, 30 mL of water added and the suspension was sonicated 15 minutes. The suspension was diluted with water, mixed well and filtrated through a Whatman no. 42 filter paper. First 10 mL of filtrate were discarded and suitable aliquots of filtrate were used for the assay of CYPHCl by the procedures described for methods A, B and C.

CONCLUSIONS

Three selective and sensitive spectrophotometric methods are proposed. The reagents used are inexpensive and easily available. The methods are unaffected by small variations in experimental conditions. Good recoveries of cyproheptadine hydrochloride and RSD values indicate that method A, B and C are accurate and precise. These methods are suitable for routine analysis of cyproheptadine hydrochloride, both in pure bulk powder and in tablets being free from interference by excipients.

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