New Methods for Determination of Clarithromycin

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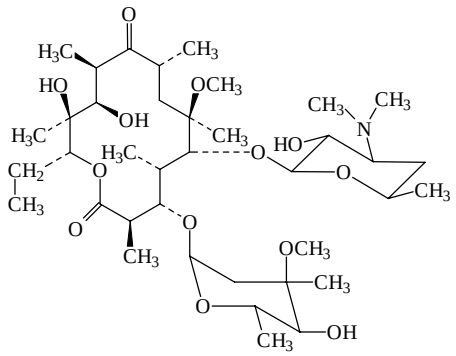
Clarithromycin is an important macrolide drug used to the recovery of many bacteriological diseases. We have observed that Clarithromycin gives very stable compounds with the chromium complex anions. Some new gravimetric, redox titrations and spectrometric methods for the determination of 6-O-methylerythromycin are described. The proposed methods are quick, fast and the statistical treatment of the experimental data shows that they are sufficiently accurate and are not affected by systematic errors.

Keywords: Clarithromycin determination, chromium complexes, analytical methods, synthesis, statistical data.

Clarithromycin or 6-O-methylerythromycin is a very important macrolide drug and active principle in many pharmaceutical products [1]. Clarithromycin is an antibiotic used to treat certain infections caused by gram-positives and gram-negatives bacteria [1-4].

Clarithromycin was synthesized by methylation of the C-6 hydroxyl group of erythromycin and has the following structure [5-7]:



Currently several methods are used for determining and dosage of Clarithromycin from pharmaceutical formulations and human body. So far, determination of clarithromycin in pure state are accomplished by some analytical techniques. Thin layer chromatography (TLC) [8,9] and high performance liquid chromatography (HPLC) [10-13] are by far the most widely used methods for determining this drug.

Electroanalytical techniques and capillary electrophoresis were also employed for the quantitative analysis of Claritromycin tablets [14-17].

Claritromycin does not have sufficient chromophoric groups, which enable this compound to be determined directly by UV-Vis spectroscopy. However, Clarithromycin forms colored compounds with some reagents, and spectrophotometric determination of this drug in pharmaceutical formulations becomes possible [18-20].

Determination of clarithromycin from biological fluids was accomplished by several methods such as: HPLC with precolumn fluorescence derivatization [21] and electrochemical detection using amperometric or coulometric detector [22,23], automated solid-phase extraction and electrochemical detection [24,25], and microbiological bioassay [26]. These techniques involved liquid-liquid extraction steps [27] and solid-phase extraction using octadecylsilica or cyano cartridges as sample cleanup procedures. Some determination methods are based on the precipitation of proteins in human serum with acetonitrile containing an internal standard and subsequently HPLC analysis [28-30].

All these methods use expensive apparatus and techniques. Previously, we synthesized several biologic actives molecules [31-33] and implemented some chemical methods for the determinations of drugs [34-36].

The aim of this study was to develop simple, rapid and reliable methods for the Clarithromycin determination using reagents and equipment available. The proposed methods are based on the reactions between Clarithromycin and complex anions of the transition metals such as Reinecke salt $NH_4[Cr(NH_3)_2(SCN)_4]\cdot H_2O$.

Experimental part

Reagents, equipment and methods

All reagents used were analytical grade: $KCr(SO_4)_2\cdot 12H_2O$, KSCN, NH_4SCN, $(NH_4)_2Cr_2O_7$, ICl, p-toluidine, Clarithromycin, CCl_4, ethanol are commercial available Fluka products; 20% HCl aqueous solution (prepared from 37% HCl, Sigma-Aldrich product), 0.1N $KMnO_4$ aqueous solution, 0.1N $KBrO_3$ aqueous solution, 0.1 N KIO_3 aqueous solution, and 3% NaOH aqueous solution were prepared from available Fluka reagents; 2% $K_3[Cr(SCN)_6]$ solution was prepared from $K_3[Cr(SCN)_6]$ synthesized [37]; $[Cr(p\text{-toluidine})_2(SCN)_4]\cdot[NH_4]^+$ was synthesized (see below ammonium di(p-toluidin) tetrakis(thiocyanato-N)chromate (1-) synthesis).

The UV-Vis spectra were recorded in 95% ethanol as a solvent using a Varian Cary® 50 UV-Vis Spectrophotometer manufactured by Agilent Technologies (Germany).

The IR/FT spectra were recorded using an ALPHA FTIR/ATR spectrometer fabricated by Bruker Optics GmbH (Germany).

Elemental analyses were carried out with a Carlo Erba model 1106 elemental analyzer produced by *Carlo Erba* (Italy). A model C-31 Cahn balance was used.

Syntheses of $K_3[Cr(SCN)_6]$ [37] and ammonium diamminetetrakis (thiocyanato-N) chromate (1-) or Reinecke salt $NH_4[Cr(NH_3)_2(SCN)_4]\cdot H_2O$ [38] were performed according to the methods of literature.

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Procedure A

Synthesis of the ion association complex of Clarithromycin.

To a beaker of 150 mL a sample of 1.16-20 mg of clarithromycin (accurately weighing ± 0.01 mg) was mixed with 20 mL of water. After addition of 4-5 mL of 20% hydrochloric acid the obtained suspension became colourless solution and clarithromycin hydrochloride made up. At room temperature, 15 mL of 2% $K_3[Cr(SCN)_6]$ aqueous solution was added. The red precipitate was filtered through a fritted-glass funnel, washed with cold distilled water until the filtrate was colourless and, finally, dried into stove at 103°C. The results of the elemental analysis of the complex were: %C=47.25 (47.27 calculated); %H=7.10 (7.12 calculated); %N=9.16 (9.12 calculated); %S=11.99 (12.00 calculated); %Cr=4.87 (4.88 calculated) - chromium was determined as Cr_2O_3 after calcination of the sample at 800°C. From these results the chemical structure of the new complex is ClarithromycinH$[Cr(NH_3)_2(SCN)_4]$.

Procedure B. Using the same experimental protocol as that described above (procedure *A*), but the anion complex $[Cr(p\text{-toluidine})_2(SCN)_4]^-$, we obtained a new compound which was separated as pall – yellow precipitate. Elemental chemical analysis are the following: %C=53.91 (calculated 53.93); %H=7.06 (7.06 calculated); %N=7.83 (7.86 calculated); %S=10.25 (10.27 calculated); %Cr=4.15 (4.17 calculated).

The chemical formula of this compound is ClarithromycinH$[Cr(p\text{-}CH_3C_6H_4NH_2)_2(SCN)_4]$.

Gravimetric determination of Clarithromycin- operating mode

Clarithromycin is precipitated in aqueous solutions with the chromium anion complexes (procedure *A* and *B*). The precipitation reaction is quantitative. The new formed precipitates are very stable and easily separated from the reaction medium by filtration, afterwards dried and, finally, weighed. From the mass of obtained precipitate is calculated the amount of drug.

Synthesis of ammonium di(p-toluidin)tetrakis(thiocyanato-N)chromate (1-).

0.1 mole of $K_3[Cr(SCN)_6]$ anhydride and 0.1 mole of p-toluidine was dissolved in methanol, then a saturated solution of NH_4Cl was gradually added until the precipitation of the compound $NH_4[Cr(p\text{-}CH_3C_6H_4NH_2)_2(SCN)_4]$. The precipitate was filtered off under vacuum, through a G_4 fritted – glass funnel, washed up with cold methanol-water solution, afterwards dried on air.

Redox titration of Clarithromycin after precipitation as ClarithromycinH$[Cr(NH_3)_2(SCN)_4]$ (A) or as ClarithromycinH$[Cr(p\text{-}CH_3C_6H_4NH_2)_2(SCN)_4]$ (B)

A sample of 2.49-19.97 mg of Clarithromycin·HCl (accurately weighing ± 0.01 mg) was precipitated from an aqueous solution in the form of the complex with Reinecke salt. The precipitate was filtered off under vacuum, through a Büchner funnel, afterwards washed 3 times with 8 mL of water, until the filtrate was colourless. Both precipitate and filter paper were transferred in a 250 mL Berzelius beaker, then the Büchner funnel was washed with 15 mL of water and, finally, with 15 mL of 3% NaOH. The precipitate of chromium (III) hydroxide became soluble adding hydrochloric acid so that the concentration of H_3O^+ ions was 1.7-2.0 M. Afterwards, 5 mL of CCl_4 and 10 drops of ICl indicator solution were added, then the mixture was titrated with 0.1 N solution of $KMnO_4$, $KBrO_3$, or KIO_3, respectively, under continuous stirring until the organic layer became colorless.

Spectrometric determination of Clarithromycin as ClarithromycinH$[Cr(NH_3)_2(SCN)_4]$

Samples containing 1.20-25.00 mg of Clarithromycin (accurately weighing ± 0.01 mg) were precipitated with a small excess of the ammonium diamminetetrakis (thiocyanato-N) chromate (1-) as ClarithromycinH $[Cr(NH_3)_2(SCN)_4]$. The precipitate was afterwards dissolved in ethanol, the solution was poured in a 50 mL volumetric flask and diluted with ethyl alcohol to the mark.

The statistical analysis of the experimental data (table 3) was achieved using linear regression method and the following equations [40, 41]:

$$\sum(x+y)^2=\sum(x^2+2xy+y^2)=\sum x^2+2\sum xy+\sum y^2$$

So $\sum(x+y)^2=1363.77$ and $\sum x^2+2\sum xy+\sum y^2=$

$=1244.55+2x58.2422+2.733724=1363.77$

$\bar{x}=10.25625$; $\bar{y}=0.48062$

$$\sigma_x=\sqrt{\frac{\sum x^2}{n}-\bar{x}^2}=7.10162;\ \sigma_y=\sqrt{\frac{\sum y^2}{n}-\bar{y}^2}=0.33301$$

$$r=\frac{\frac{\sum xy}{n}-\bar{x}\bar{y}}{\sigma_x\sigma_y}=0.9941\approx 1$$

$$y-\bar{y}=r\frac{\sigma_y}{\sigma_x}(x-\bar{x});\ y=0.046615x+0.00252$$

$$x-\bar{x}=r\frac{\sigma_x}{\sigma_y}(y-\bar{y});\ x=21.997y+0.06723$$

Results and discussions

In order to prove the formation of an ion-association between Clarithromycin and Reinecke salt we performed IR spectra of the initial reagents and the resulting product. The spectra were recorded in the 500-4000 cm^{-1} range to determine the chemical structure of the compounds.

The IR spectrum of ammonium diamminetetrakis (thiocyanato-N) chromate (1-), exhibits a very intense peak at 2109 cm^{-1} and a peak at 2045 cm^{-1}, both correspond to the Ca≡N stretching vibrations from thiocyanato group SCN^-. A large absorption band with a medium intensity occurs at 3000-3294 cm^{-1} and is assigned to the O-H stretching vibrations from H_2O and N-H stretching vibrations of NH_3 molecules.

Infrared spectrum of Clarithromycin exhibits stretching characteristic vibration bands due to carbonyl group at 1690 cm^{-1}, ester function at 1732 cm^{-1} and hydroxyl group at 3460 cm^{-1}. The ether function C-O-C shows infrared absorption peaks at 940 cm^{-1} and 1160 cm^{-1}. The C-N bond of tertiary amine group appears in the fingerprint region at 1033 cm^{-1} and is generally not useful for functional group identification [39].

IR spectrum of the complex Clarithromycin $H[Cr(NH_3)_2(SCN)_4]$ is shown in figure 1. The main IR absorption bands of the complex are: 2914 cm^{-1}, 2078 cm^{-1}, 1695 cm^{-1}, 1018 cm^{-1} and 718 cm^{-1}. The peak of carbonyl group from this complex shows up at 1695 cm^{-1} and the peak of Ca≡N stretching vibration from thiocyanato group at 2078 cm^{-1}, both with medium intensity. Two intense peaks appear at 1018 cm^{-1} and 1102 cm^{-1} and are due to ether functional group. The IR spectrum of compound exhibits two important peaks of medium intensity at 2914 cm^{-1} and 1463 cm^{-1}. These peaks are due

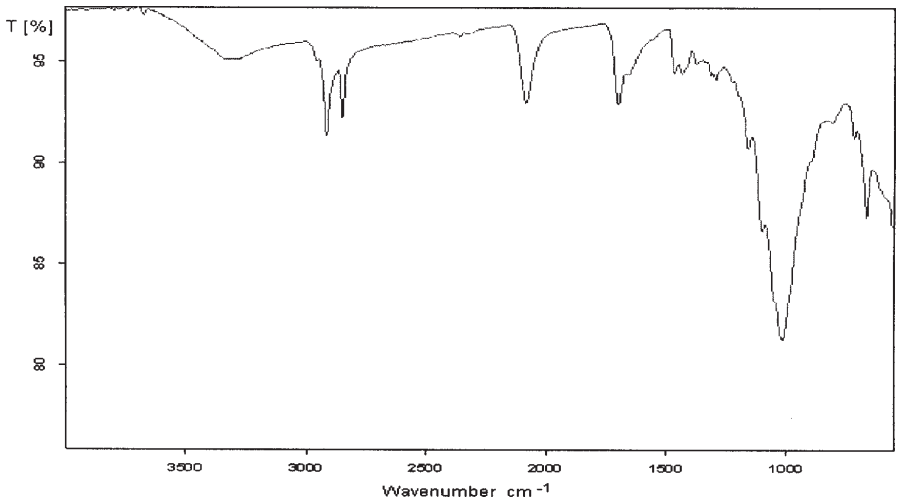

Fig. 1. IR spectrum of ClarithromycinH$[Cr(NH_3)_2(SCN)_4$

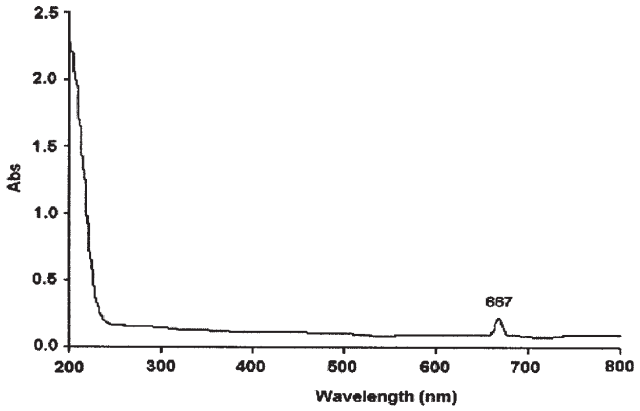

Fig. 2. UV-Vis spectra of ClarithromycinH$[Cr(NH_3)_2(SCN)_4]$ (II) in EtOH

to quaternary ammonium group issued from the reaction of Clarithromycin with Reinecke salt.

UV-Vis spectrum of Clarithromycin in ethanol shows no absorption band in the range of 200-800 nm. In contrast, the UV-Vis spectrum of the complex ClarithromycinH $[Cr(NH_3)_2(SCN)_4]$ exhibits an absorption band in the visible region at 667 nm (fig. 2). This band is used in this paper to determine Clarithromycin spectrophotometrically by Vis absorption of the complex.

Gravimetric determination of Clarithromycin

Gravimetric determination of Clarithromycin ($G_{complex}$) was made after precipitation as ClarithromycinH $[Cr(NH_3)_2(SCN)_4]$ (by procedure *A*) or ClarithromycinH $[Cr(p\text{-}CH_3C_6H_4NH_2)_2(SCN)_4]$ (by procedure *B*). The experimental results were interpreted statistically (table 1). We can see that this method of determination of Clarithromycin is not affected by systematic errors.

Redox titration of Clarithromycin

Oxidimetric determination of Clarithromycin by titration was effectuated after its precipitation as ClarithromycinH$[Cr(NH_3)_2(SCN)_4]$ (*A*) or ClarithromycinH $[Cr(p\text{-}CH_3C_6H_4NH_2)_2(SCN)_4]$ (*B*). Three redox titrations methods: permanganometric, bromatometric and iodatometric methods based on the redox reactions between SCN^- and the oxidizing agent MnO_4^-, BrO_3^-, and IO_3^-, respectively have been developed. Iodine monochloride, ICl/CCl_4, was used as redox indicator.

The experimental data obtained by redox titration determinations of Clarithromycin are presented in table 2. The redox titration determinations are not affected by systematic errors and is quite accurate.

Spectrometric determination of Clarithromycin

Spectrometric determination of Clarithromycin was made after being converted into ClarithromycinH

Table 1

GRAVIMETRIC DETERMINATION OF CLARITHROMYCIN AS CLARITHROMYCIN**H**$[Cr(NH_3)_2(SCN)_4]$ (PROCEDURE *A*) AND CLARITHROMYCIN**H**$[Cr(p\text{-}CH_3C_6H_4NH_2)_2(SCN)_4]$ (PROCEDURE *B*)

Clarithromycin mg taken	Gravimetric determination							
	A				B			
	Weighed $G_{complex}$	Clarithromycin found mg	Error mg	Error %	Weighed $G_{complex}$	Clarithromycin found mg	Error mg	Error %
1.16	1.65	1.15	-0.01	0.86	1.95	1.17	+0.01	0.85
4.31	6.11	4.28	-0.03	0.70	7.22	4.33	+0.02	0.46
6.97	9.92	6.95	-0.02	0.28	11.57	6.94	-0.03	0.403
9.58	13.74	9.62	+0.03	0.31	15.88	9.52	-0.07	0.73
11.76	16.87	11.82	+0.06	0.50	19.77	11.85	+0.09	0.75
13.88	19.68	13.79	-0.09	0.65	23.07	13.83	-0.05	0.36
17.62	24.99	17.57	-0.11	0.62	29.63	17.76	+0.14	0.78
20	28.72	20.12	+0.12	0.59	33.43	20.04	+0.04	0.19
	$\overline{X}$ =9.58 S^2=4.56·10^{-4} S=2.13·10^{-2} t=2.26 $t_{n-1,\alpha}$=2.37; α=95% $\overline{X}$ -ts<A< $\overline{X}$ +ts 9.53<9.59<9.62				$\overline{X}$ =17.61 S^2=7.03·10^{-4} S=2.65·10^{-2} t=2.26 $t_{n-1,\alpha}$=2.26; α=95% $\overline{X}$ -ts<A< $\overline{X}$ +ts 17.55<17.62<17.66			

Obs.: n=8 determinations.

Clarithromycin·HCl taken mg	$\overline{X}$ mg	S	t_a	t_b	$t_{n-1,\alpha}$ α=95%
Permanganometric determination					
2.49	2.500	$1.73 \cdot 10^{-2}$	$3.586 \cdot 10^{-4}$	$2.045 \cdot 10^{-2}$	2.23
14.97	14.983	$2.77 \cdot 10^{-2}$	$8.984 \cdot 10^{-4}$	$3.487 \cdot 10^{-2}$	2.23
Bromatometric determination					
7.50	7.509	$1.89 \cdot 10^{-2}$	$4.987 \cdot 10^{-4}$	$2.984 \cdot 10^{-2}$	2.23
19.97	19.982	$2.66 \cdot 10^{-2}$	$5.691 \cdot 10^{-4}$	$3.854 \cdot 10^{-2}$	2.23
Iodatometric determination					
4.99	5.00	$2.41 \cdot 10^{-2}$	$2.964 \cdot 10^{-4}$	$1.577 \cdot 10^{-2}$	2.23
17.47	17.478	$2.63 \cdot 10^{-2}$	$4.562 \cdot 10^{-4}$	$3.691 \cdot 10^{-2}$	2.23

Obs.: 1 mL of 0.1 N solution of MnO_4^-, BrO_3^-, IO_3^- is equivalent to 1 mg Clarithromycin; n=10 determinations.

Table 2
OXYDIMETRIC DETERMINATION OF CLARITHROMYCIN AS CLARITHROMYCIN**H**$[Cr(NH_3)_2(SCN)_4]$

N	x mg	x^2	y	y^2	xy	x+y	$(x+y)^2$
1	0.57	0.32	0.026	0.000676	0.0148	0.596	0.3552
2	1.32	1.74	0.062	0.003844	0.0818	1.382	1.909
3	4.97	24.70	0.233	0.054289	1.1580	5.203	27.0712
4	9.56	91.39	0.448	0.200704	4.2829	10.008	100.1600
5	12.23	149.66	0.573	0.328329	7.0077	12.803	163.9168
6	14.17	200.87	0.664	0.440896	9.4088	14.834	220.0475
7	17.83	317.90	0.836	0.698896	14.9058	18.666	348.4195
8	21.40	457.96	1.003	1.006009	214642	22.403	501.8944
Total	82.05	1244.55	3.845	2.733724	58.2422	85.895	1363.7745

Obs. Samples of 25 mL

Table 3
SPECTROPHOTOMETRIC DETERMINATION OF CLARITHROMYCIN AS CLARITHROMYCIN**H**$[Cr(NH_3)_2(SCN)_4]$

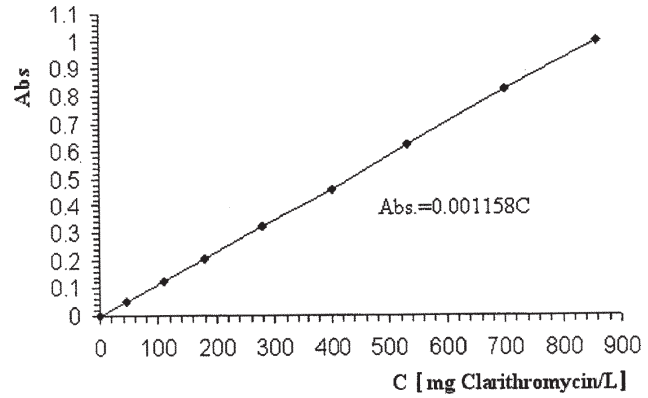

Fig. 3. Calibration curve for the spectrometric determination of Clarithromycin as ClarithromycinH $[Cr(NH_3)_2(SCN)_4]$

$[Cr(NH_3)_2(SCN)_4]$. Visible absorption maximum at λ=667 nm of the complex ClarithromycinH$[Cr(NH_3)_2(SCN)_4]$ was used. The Lambert-Beer's law is followed in the 0.0231-0.8562 mg/mL range. The statistic interpretation of the experimental data was accomplished by means of linear regression method [40, 41] and the obtained results are shown in the table 3.

The linear dependence of the absorbance versus the concentration of Clarithromycin is shown in the figure 3. The angle between the two straight lines was very small, r=0.9941, the dependence between the absorbance and the concentration of Clarithromycin was linear, Abs.=0.001158C.

Conclusions

Quantitative determination of Clarithromycin can be performed by three different new procedures based on gravimetric, spectrometric and redox titration methods. The determination methods are very easy and the sensibility of the reactions allows the dosage a few quantities of compound. Following the statistical interpretation of the experimental data it results that the proposed methods were not affected by systematic errors and were quite accurate. These methods can be employed by the medical laboratories of analyze and control.

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