A QUANTITATIVE METHODOLOGY FOR DETERMINATION OF IMIDAZOLIC COMPOUNDS IN PHARMACEUTICAL CREAMS, UTILIZING A COBALT COMPLEX (II) ASSAY

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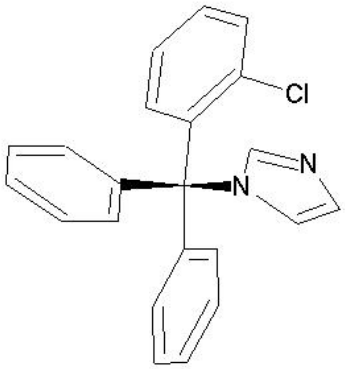
1. Summary:

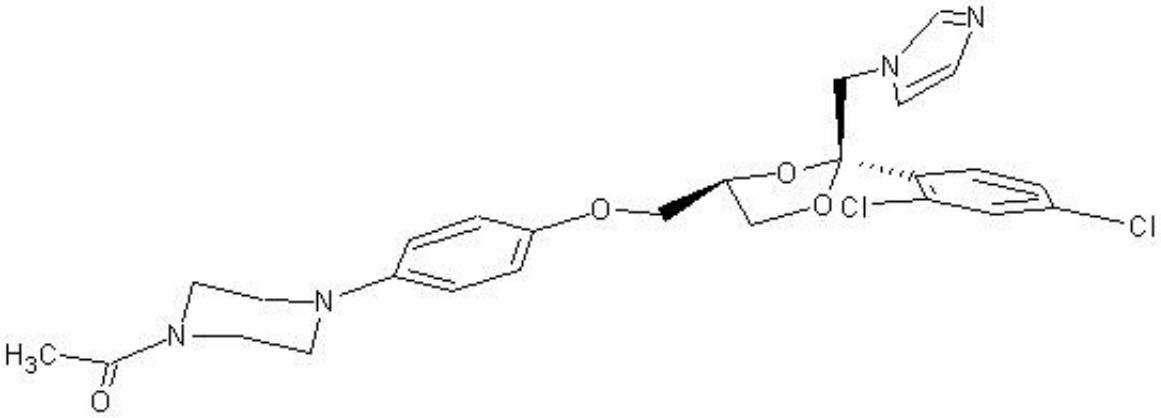
Imidazole antimycotics are widely used mainly in topical drug formulations, for manufacture of pharmaceutical creams such as Clotrimazole and Ketoconazole. USP-XXV and BP-2001 pharmacopoeias describe HPLC assays for Clotrimazole while a method for Ketoconazole Cream is not included.
An innovative method has been established based upon a colored complex formed by the reaction of Cobalt (II) and the imidazole side chain of these fungicides, which can be quantified via visible spectroscopy. Though **not** Stability Indicating, this assay does provide a rapid and reliable technique for in-process control. The theoretical concepts of the method are highlighted and the detailed methodologies for quantification of Clotrimazole and Ketoconazole in pharmaceutical creams are given.

2. Introduction

Imidazole antimycotics, as Clotrimazole and Ketoconazole, are weak organic bases, with strong steric hindering at the basic function and the three phenyl groups make Clotrimazole a very lipophylic substance.



Clotrimazole



Ketoconazole

Typical formulations of pharmaceutical creams on the market consist of the active ingredient and excipients such as essential oils, preservatives like parabens, fats and emulsifiers in aqueous or polar non-aqueous solvents as Propylene Glycol and Ethanol.

The quantitative assays for Clotrimazole and Ketoconazole in different drug formulations, described in the USP-XXV and BP-2001 pharmacopoeias are realized by HPLC.

Methods employing double extraction procedures present their well known disadvantages and a volumetric assay described in USP XX for Clotrimazole cream, using Sodium Laurylsulfate titration, is time consuming and includes a not easy detectable end point.

An innovative method has been established, based on an aquamarine colored complex reaction of Cobalt (II) and the imidazole side chain of these fungicides, which can be quantified via visible spectroscopy. Though **not** Stability Indicating, this assay does provide a rapid and reliable technique for in-process control, specially for companies with few resources.

3. Theoretical concepts

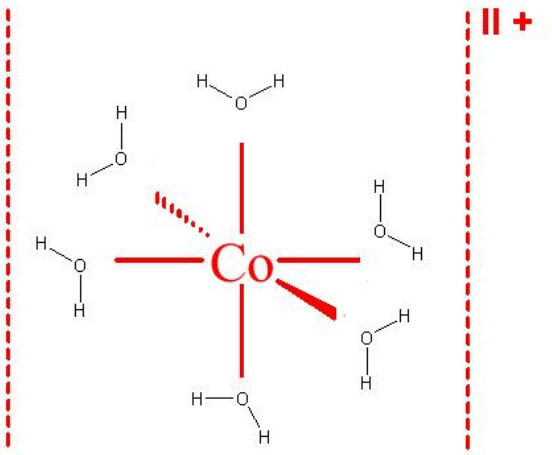
Cobalt in its oxidation state 2^+, (II), as e.g. in $CoCl * 6H_2O$, has 7 d-electrons , which determine its chemical properties. Cobalt (II) forms ionic complexes with different ligands, mainly the octahedral or tetrahedral coordination. Possible examples for ligands include Water, Chloride or as in this method, the imidazolic side chain of Clotrimazole or Ketoconazole.

For no other transition metal, the difference in the stability of octahedral and tetrahedral complexes is so small as in the case of Cobalt (II). Even it exists an equilibrium of the two geometric structures of the complex, e.g. for water as ligand (in water solution):

$$[Co(H_2O)_6]^{2+} \rightleftharpoons [Co(H_2O)_4]^{2+}$$

The color of these complexes changes with the coordination (and the type of the solvent): The octahedral complexes are pink colored. The tetrahedral complexes are blue colored.

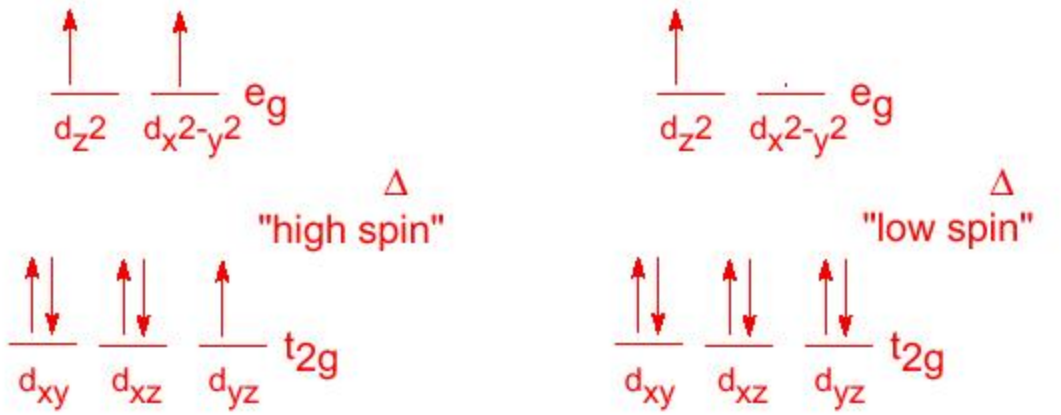
Hexa-aqua-complex of Co (II) in water:



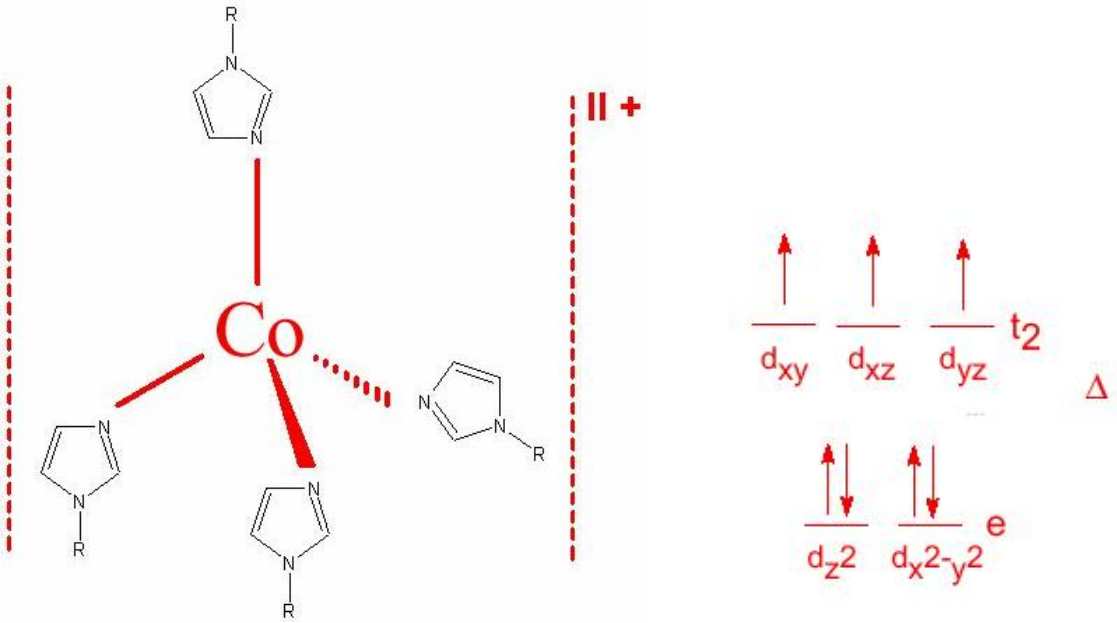
In polar but anhydrous solvents, these two complexes are well defined and differentiated, according to the type of ligand.

The spectroscopic, magnetic and color characteristics are given by the d-electrons, respectively by the electronic configuration of the Molecular Orbital (MO) from the complex.

The octahedral complex of $[Co(H_2O)_6]^{2+}$ has the following structure and electronic configuration for the d-orbitales. The energy difference of the Crystal Field Splitting in high- and low-spin complexes is plotted on **Y-**axis, and their electronic configuration with the symmetry group of the energy transition levels in the MO is also indicated:



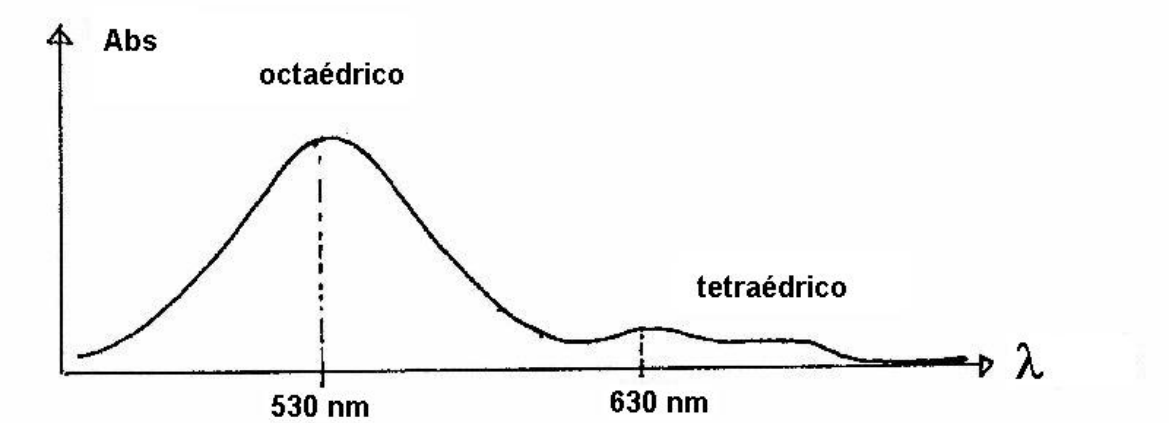
In the case of Cobalt (II), the energy splitting of the d-orbitales is small, compared with other complexes (e.g. Ni, Fe) and $[Co(H_2O)_6]^{2+}$ is a high-spin complex (three unpaired electrons; the low-spin complex has one unpaired electron; see Pauli and Hund's rules). The difference in CFS-energy (Crystal Field Stabilization) of the octahedral and tetrahedral complex is small. With stronger ligands than water, e.g. with the imidazole ring of Clotrimazole or Ketoconazole, the coordination changes from octahedral to tetrahedral:



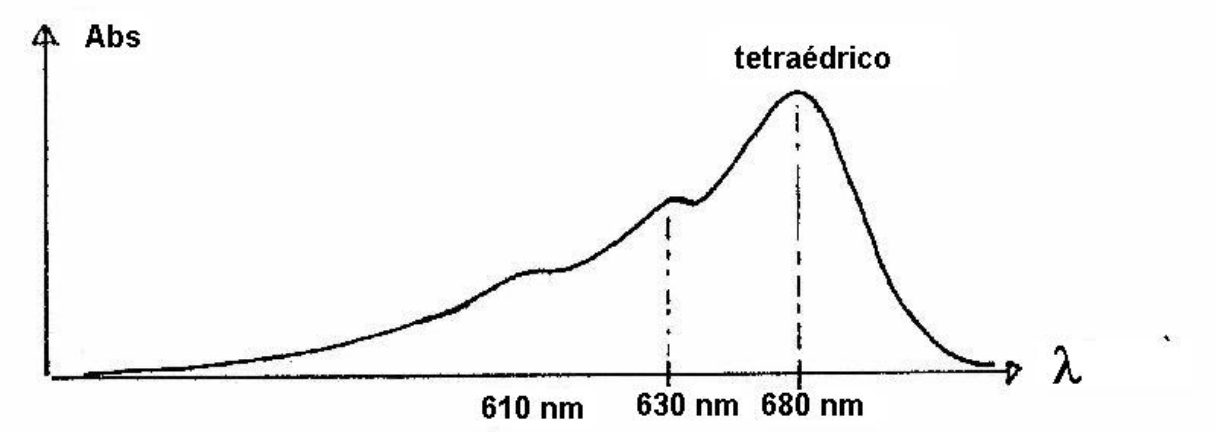
It's beautiful that these changes in molecular structures, caused by complex electronic phenomena, can be observed by visible spectroscopy and therefore by eye, changing its color from pink to aquamarine.

4. Spectroscopic properties

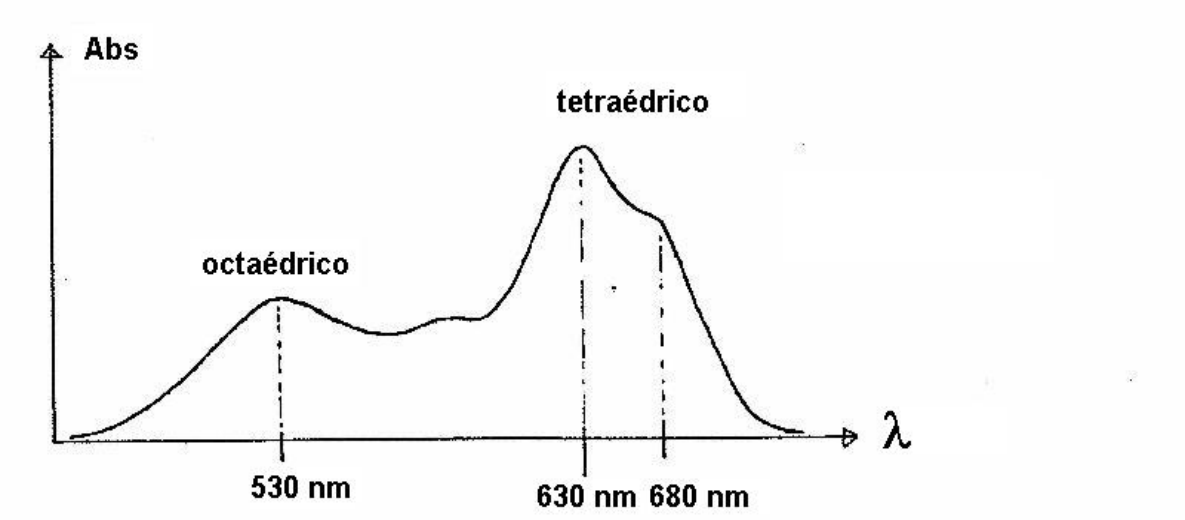
The optimal solvent mixture we have found for Ketoconazole and Clotrimazole contains 10 ml of Chloroform (81.3%), 2 ml of Ethanol (16.3%) and 0,3 ml of Acetone (2.4%), the last one also contains the Cobalt Chloride (II) Hexahydrate.
The $[Co(H_2O)_6]^{2+}$ complex appears in this solvent in octahedral coordination with water (**pink color**) and in a low concentration in the tetrahedral coordination:



In pure acetone, the $[Co(H_2O)_6]^{2+}$ complex appears in tetrahedral coordination with water (**blue, aquamarine color**).



When Clotrimazole or Ketoconazole is added to the solvent, these both completely form the tetrahedral complex with Cobalt (II). The Cobalt (II) leftover, stays in octahedral coordination with water.



As it can be observed, the wavelengths maximums of the octahedral and tetrahedral complexes are well separated by approximately 100 nm.
The equilibrium of the complexes in solution can be written as follows:

$[Co(H_2O)_6]^{2+}$ D $[Co(H_2O)_4]^{2+}$ D $[Co(H_2O)_2(\text{R-imidazole})_2]^{2+}$ D $[Co(\text{R-imidazole})_4]^{2+}$

pink **aquamarine or blue**

The wavelength maximum for the tetrahedral complex in the solvent mixture after the extraction (Chloroform, Ethanol, Acetone), is reproducibly at 577 nm, more hypochromic than in Acetone, due to Chloroform and a low concentration of water. The wavelengths maximums of the octahedral and tetrahedral complexes in the solvent mixture after the extraction, are still separated by approximately 47 nm.

These complexes are kinetically unstable, therefore it is necessary to take into account the time since their formation, to obtain reproducible values for the spectroscopic absorptivity.

In the visible part of the spectrum dominates the electronic transition of the quartet spin configuration.

In the octahedral case it is the electronic transition

(**triplet-triplet**) $^4T_{1g(F)} \longrightarrow {}^4T_{1g(P)}$ observed approximately at 530 nm,

and in the tetrahedral case it is the electronic transition

(**duplet-triplet**) $^4A_{2(F)} \longrightarrow {}^4T_{1(P)}$ observed approximately at

610nm, 630 nm and 680 nm, with three transitions, due to spin-orbit coupling. In the solvent mixture the maximums are shifting hypochromically about 50 nm. The complex with Cobalt (II) develops its maximum absorbance and reproducibility after 10-15 minutes, calculating the concentration of Clotrimazole or Ketoconazole as active ingredients of the pharmaceutical cream.

The interference of water has to be considered, because it competes with Clotrimazole and Ketoconazole in the formation of the complexes. But in the solvent mixture (Chloroform, Ethanol, Acetone), the concentration of the water is constant and well-known ($6H_2O$ in the Cobalt salt and 5% in Ethanol). In a cream with a given drug formulation the water concentration is constant too. The developed system presents **robustness.**

5. Analytical method for Clotrimazole and Ketoconazole Cream

5.1. EQUIPMENT REQUIRED	CAPACITY
Test tube	-
Volumetric pipettes	2, 10, 20 ml
Volumetric flasks	10, 25 ml
Serological pipettes	1, 5 ml
Separator funnel	60 ml
Test tube	25 ml

5.2. REAGENTS REQUIRED	CONCENTRATION
Distilled water	-
Chloroform	RG
Cobalt Chloride Hexahydrate	300 mg $CoCl_3$*6 H_2O, dissolved in 10 ml Acetone
Ethanol	95%

5.3. Assay:

PRODUCT: **Clotrimazole-cream**
PRESENTATION: **CLOTRIMAZOLE, 1%**

Preparation: Accurately weigh 4.00 g of Clotrimazole cream, in a separator funnel. Add with volumetric pipette 20 ml of Chloroform followed by 20 ml of distilled water. Shake thoroughly several times and allow to stand 15-20 minutes for good phase separation. Carefully withdraw to avoid passage of the greasy inter-phase (this depends on the amount of emulsifiers and greasy components used in the drug formulation), approximately 15 ml of the chloroformic phase into a test tube.
Transfer 10 ml with a volumetric pipette to a 25 ml volumetric flask, add 2 ml of Ethanol and 3% (w/v) of $CoCl_3$*6 H_2O dissolved in 0,3 ml Acetone. An intense **aquamarine** color is produced. (Do not fill the flask). Shake recording the time, after 8

minutes, remove an aliquot to the UV cell. Wait for 10-15 minutes to plot the spectrum with maximum detection. Use the solvent mixture without Cobalt as the Blank.

PROCEDURE: Plot the spectrum with maximum detection.
Blank, Chloroform
Mode: Absorbance
Limits: -0.01, 1.3 (Abs)
Wavelength range: 500 nm and 800 nm
Speed: 200-400 nm/min
Response: Medium (= slit of 2 nm)

Report the absorbance at a wavelength of **577.2 nm**.

CALCULATION:

$$\% \text{ CLOTRIMAZOLE} = \frac{\mathbf{Abs_C \times 100\%}}{\mathbf{0.735}}$$

Abs. = 0.735 = Std., it could be normalized again, if it would be necessary
Abs_C: Absorbance of the CLOTRIMAZOLE-COBALTO (II) complex at 577.2 nm
Specification for CLOTRIMAZOLE: 90% - 110%

PRODUCT: KETOCONAZOLE -cream
PRESENTATION: KETOCONAZOLE, 2%

Preparation: Accurately weigh 3.00 g of Ketoconazole cream, in a separator funnel. Add with volumetric pipette 20 ml of Chloroform followed by 20 ml of distilled water. Shake thoroughly several times and allow to stand at least 30 minutes for good phase separation. Very carefully withdraw to avoid passage of the greasy inter-phase (this depends on the amount of emulsifiers and greasy components used in the drug formulation), approximately 15 ml of the chloroformic phase into a test tube.
Transfer 10 ml with a volumetric pipette to a 25 ml volumetric flask, add 2 ml of Ethanol and 3% (w/v) of $CoCl_3$*6 H_2O dissolved in 0,3 ml Acetone. An intense **aquamarine** color is produced. (Do not fill the flask). Shake recording the time, after 8 minutes, remove an aliquot to the UV cell. Wait for 10-15 minutes to plot the spectrum with maximum detection. Use the solvent mixture without Cobalt as the Blank.

P.D: Cobalt (II) solution in Acetone can be stored for several weeks in low actinic glassware, if necessary 5-10 mg of anhydrous Sodium Sulphate can be added.

PROCEDURE: Plot the spectrum with maximum detection.
Blank, Chloroform
Mode: Absorbance
Limits: -0.01, 1.3 (Abs)
Wavelength range: 500 nm and 800 nm
Speed: 200-400 nm/min
Response: Medium (= slit of 2 nm)

Report the absorbance at a wavelength of **576.8 nm**.

CALCULATION:

$$\% \text{ Ketoconazole} = \frac{\mathbf{Abs_C \times 100}\%}{\mathbf{0.715}}$$

Abs. = 0.715 = Std., it could be normalized again, if it would be necessary
Abs_C: Absorbance of the Ketoconazole-COBALTO (II) complex at 576.8 nm
Specification for Ketoconazole: 90% - 110%

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6. References

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