Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 06, pp.16805-16810, June, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

SIMULTANEOUS HPLC UV-VIS IDENTIFICATION AND QUANTIFICATION OF CYANOCOBALAMINE (VITAMINE B12), BENZYL ALCOHOL, CHLORAMPHENICOL, PREDNISOLONE, TYLOSIN A, TYLOSIN B, TYLOSIN C AND TYLOSIN D IN FINISHED PHARMACEUTICAL PRODUCT C.T.P.12.

***Maria Neagu and Gabriela Mazilu**

Research and Development, Department of SC Pasteur Filiala Filipesti SRL, Romania

ARTICLE INFO

Article History:
Received 26th March, 2015
Received in revised form
15th April, 2015
Accepted 26th May, 2015
Published online 27th June, 2015

Key words:

HPLC, Cyanocobalamine,
Benzyl alcohol,
Chloramphenicol,
Prednisolone,
Tylosin tartrate,
Validation.

ABSTRACT

This study aims was simultaneous identified and quantified, used a HPLC method, eight active pharmaceutical ingredients, Cyanocobalamin (Vitamine B12), Benzyl alcohol, Chloramphenicol, Prednisolone, Tylosin A, Tylosin B, Tylosin C and Tylosin D. In a very short time, and with very low cost, all eight compounds was separated and quantified used this HPLC method (UV-VIS detection). The method is enough sensitive to quantified and monitorised related substances/degradation product according to VICH GL 11(R). The method can be easily applied in any analytical laboratory, because it uses common reagents, a chromatographic column frequently used, octadecylsilylsilicagel (C18) and common equipments in pharma laboratories (HPLC, multiwavelengths UV-VIS detection).

Copyright © 2015 Maria Neagu and Gabriela Mazilu. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

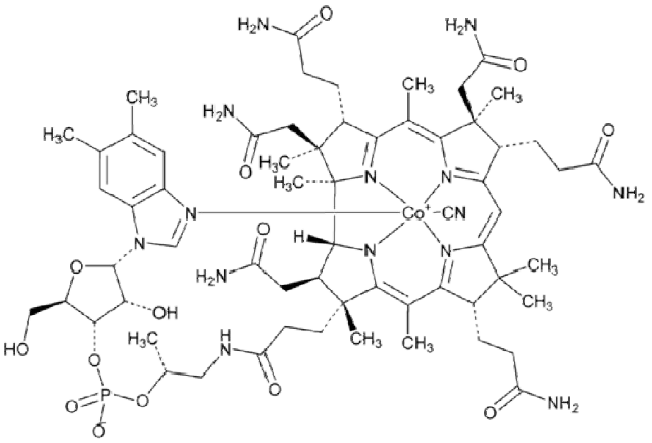
Citation: Maria Neagu and Gabriela Mazilu, 2015. "Simultaneous HPLC UV-VIS identification and quantification of cyanocobalamine (Vitamine B12), Benzyl alcohol, Chloramphenicol, Prednisolone, Tylosin A, Tylosin B, Tylosin C and Tylosin D in finished Pharmaceutical product C.T.P.12.", *International Journal of Current Research,* 7, (6), 16805-16810.

INTRODUCTION

The purpose of this study was to developed and validated the analytical method HPLC for identified and quantified the level of Cyanocobalamine, Benzoic acid, Chloramphenicol, Prednisolone and Tylosin Tartrate(Sum of Tylosin A, Tylosin B, Tylosin C and Tylosin D), related substances/degradation products in C.T.P. 12, finished pharmaceutical product. All of eight active pharmaceutical ingredientsseparated with this method are very using in veterinary treatments. Vitamin B12, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid metabolism andamino acid metabolism (Yamada, Kazuhiro, 2013).

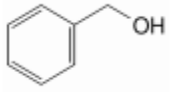
Corresponding author: Maria Neagu ,
Research and Development Department of SC Pasteur Filiala Filipesti SRL, Romania.

1. Molecular structure (United Stated Pharmacopoeia):



Molecular formula: $C_{63}H_{88}CoN_{14}O_{14}P$ (United Stated Pharmacopoeia), Molecular Weight (United Stated Pharmacopoeia): 1355.37 g/mol;

Benzyl alcohol–is used in pharmaceutical industry as antimicrobial preservative, disinfectant or as solvent.
Structural Formula:



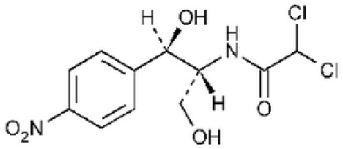
Molecular formula: C_7H_8O; Molecular Weight: 108.14 g/mol
Chloramphenicol (INN) is an antibiotic useful for the treatment of a number of bacterial infections.

Chloramphenicol, also known as chlornitromycin, is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms (Falagas *et al*., 2008).

Although its use in veterinary medicine is highly restricted, chloramphenicol still has some important veterinary indications (Chloramphenicol and Congener, 2014).

Chloramphenicol has a broad spectrum of activity of bacteriaHaemophilus influenza, Staphylococcus aureus, Streptococcus pneumoniae,Neisseria meningitidis septicaemia, Escherichia coli, Rocky Mountain spotted fever, Enterococcus faecium. It is not effective against Pseudomonas aeruginosa (http://antibiotics.toku-e.com/antimicrobial_507.html).

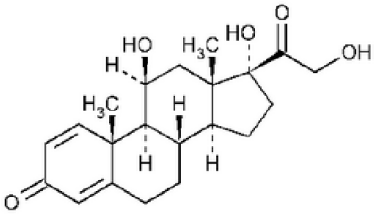
Molecular structure:



Molecular formula:$C_{11}H_{12}Cl_2N_2O_5$; Molecular weight: 323.13;
Prednisolone is used for treat of more inflammatory and auto-immune disease.

It is a derivat of cortisol, a synthetic glucocorticoid.

Molecular structure:



Molecular formula:$C_{21}H_{28}O_5$(anhydrous); Molecular weight: 360.45; Tylosin tartrate (Sum of Tylosin A + Tylosin B + Tylosin C + Tylosin D)

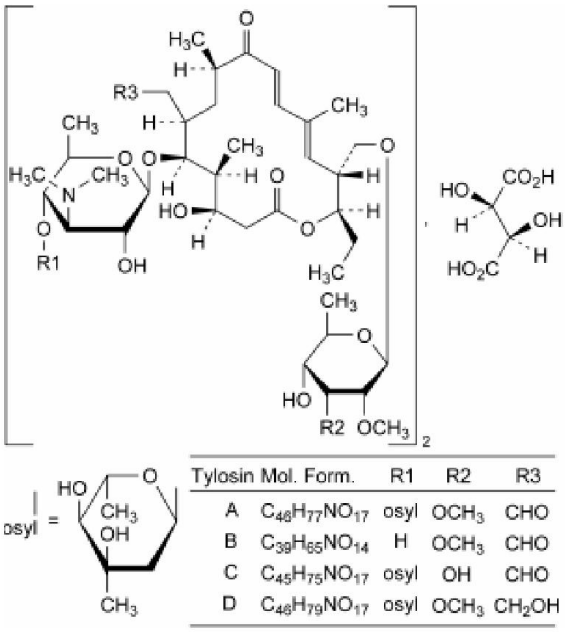
An antibiotic with a large macrocyclic lactone ring Tylosin tartrate for veterinary use is a tartrate of a mixture of macrolide antibiotics produced by a strain of Streptomyces fradiae or by any other means. The main component of the mixture is (4R,5S,6S,7R,9R,11E,13E,15R,16R)-15-[[(6- deoxy-2,3-di-O-methyl-β-D-allopyranosyl)oxy]methyl]-6-[[3,6- dideoxy-4-O-(2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-diene-2,10-dione (tylosin A, tartrate Mr 1982). Tylosin B (desmycosin, tartrate Mr 1694), tylosin C (macrocin, tartrate Mr 1954) and tylosin D (relomycin, tartrate Mr 1986) may also be present (European Pharmacopoeia).

It has a broad spectrum of activity against Gram-positiveorganisms and a limited range of Gram-negative organisms (Steeve Giguere and Dowling, ?). It is a macrolide antibiotic.

It is used in veterinary medicine to treat bacterial infections in a wide range of species and has a high margin of safety (^ Jump up to:[a] [b] Tylan 200 Injectable). It has also been used as a growth promotant in some species, and as a treatment for colitis in companion animals (^ Jump up to:[a] [b] [c] [d] Tylosin).

Tylosin tartrate […] is commonly used in a variety of animals, including dogs, cats, cattle, horses and more (www.vetinfo.com/tylosin-tartrate-for-dogs.html).

Molecular structure:



Tylosin	Mol. Form.	R1	R2	R3
A	$C_{46}H_{77}NO_{17}$	osyl	OCH_3	CHO
B	$C_{39}H_{65}NO_{14}$	H	OCH_3	CHO
C	$C_{45}H_{75}NO_{17}$	osyl	OH	CHO
D	$C_{46}H_{79}NO_{17}$	osyl	OCH_3	CH_2OH

[European Pharmacopoeia]

Molecular Weight: 1066.19, Molecular Formula: $C_{46}H_{77}NO_{17}•C_4H_4O_6$

MATERIALS AND METHODS

The method rationale is to introduce a single chromatographic procedure HPLC to identify/quantify active pharmaceutical ingredients and monitor related compounds/degradation products in finished pharmaceutical product C.T.P.12 manufactured by SC PASTEUR FILIALA FILIPESTI SRL, FILIPESTII DE PADURE, PRAHOVA, STRADA PRINCIPALA, No 944, ROMANIA.

The method is investigated, according to ICH/VICH guidelines.

Method description

Chemicals used: Tylosin Phosphate for peak identification - Reference Substance, EDQM, batch 1, Tylosin – Reference Substance, EDQM, batch 2,Tylosin D– Reference Substance, EDQM, batch 2, Tylosin tartrate, lot C91131036, NINGXIA TAIRUI Pharmaceutical Co., LTD, Chloramphenicol– Reference Substance, EDQM, batch 3, Vitamin B12 – Reference substance, EDQM, batch 5, Prednisolone– Reference Substance, EDQM, batch 8, Acetonitrile – Reagent grade, MERCK, batch I741630, Sodium perchlorate – Reagent grade, MERCK, batch A0774264, Ultrapurified water (0.55µS/cm), Benzyl Alcohol, lot 4K29AE1, NEOS CHLOROTOLUENES

Chromatographic conditions: HPLC AGILENT 1260INFINITY (UV-VIS detection), Elution mode: Gradient, Chromatographic Column:KROMASIL, C18, 250 x 4.6 mm, 5 µm, Column temperature: 60^0C,Injection volume:4 µL, Detection:at λ=254 nm for Tylosin C, Tylosin B, Tylosin D, Tylosin A, Prednisolone, Benzyl alcohol and at λ=361nm for Cyanocobalamine (Vitamine B 12) and Chloramphenicol.Mobile Phase: Solvent A: 200.0 g/L of sodium perchlorate R adjusted to pH=2.5 with 1M hydrochloric acid; Solvent B: Acetonitrile

Table 1. Gradient elution

Time, min	Solvent A	Solvent B	Flow, ml/min
0 - 4	77	23	0.8
4-8	74	26	4.0
8-20	55	45	4.0
20-23	55	45	0.8
23-30	77	23	0.8

Standard Solutions preparation

Solvent mixture for samples/ standards preparation: Ultrapurified water : Acetonitrile = 50:50 (V/V).

Reference solution for assay/ Assay sample:100000 ppm Chloramphenicol, 27500 ppm Tylosin tartrate,2500 ppm Prednisolone, 50 ppm Cyanocobalamine, 5000 ppm Benzyl alcohol.

Reference solution for related substances/degradation product:1000 ppm Chloramphenicol.

Sample solution for quantified/monitorised related substances/ degradation product are the same with Assay sample.

System suitability conditions was

In chromatogram recorded for assayreference solution, at 254nm, the resolution between Tylosin A, Tylosin B, Tylosin C and Tylosin D peaks must be minimum 1.5, symmetry factor for peaks of Tylosin A, Tylosin B, Tylosin C and Tylosin D, must be maximum 2 and resolution must be minimum 18 between the peak of Benzyl Alcohol and Prednisolone and minimum 30 between Prednisolone and Tylosin C. In chromatogram recorded for assayreference solution, at λ=360 nm, the resolution between Cyanocobalamine and Chloramphenicol must be minimum 30 and symmetry factor for Chloramphenicol must be maximum 2. The parameters (retention time, resolution, symmetry factor) was calculated used OpenLab A.01.05 software.

Peaks identification

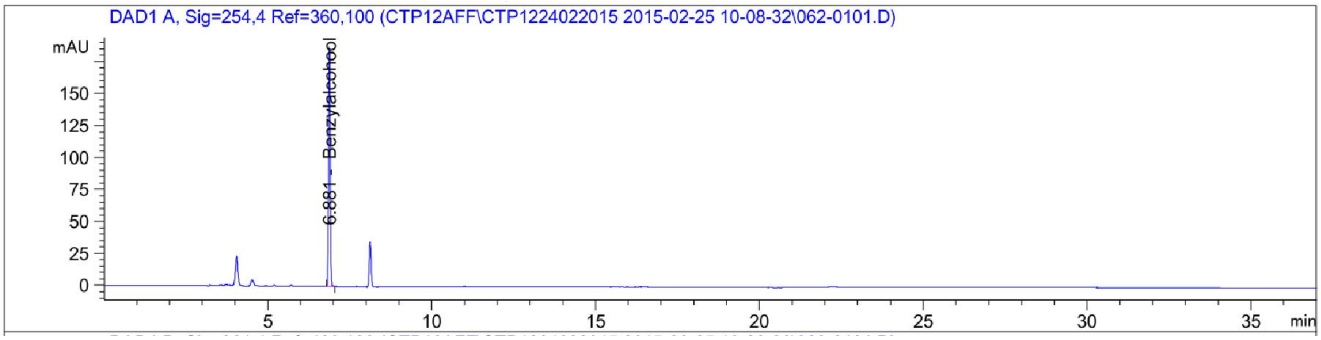


Fig. 1. Representative chromatogram for benzyl alcohol at λ= 254 nm

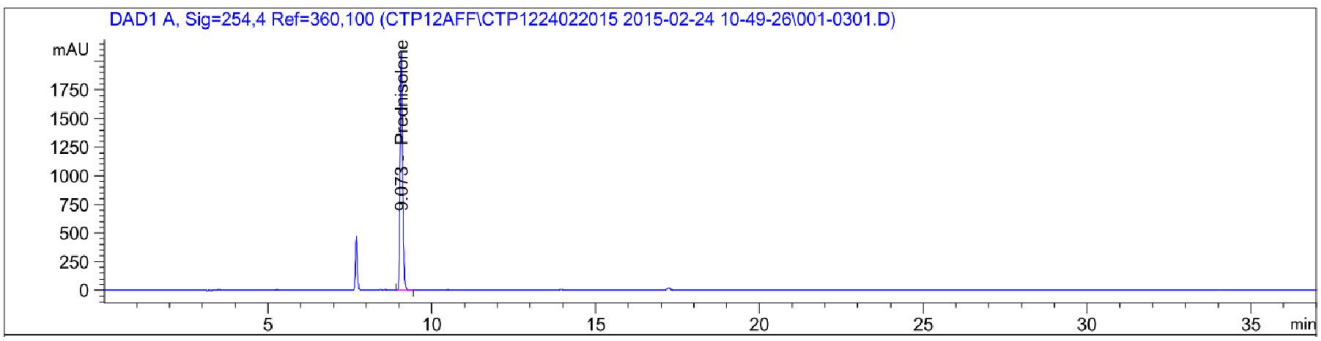


Fig. 2. Representative chromatogram for Prednisolone at 254 nm

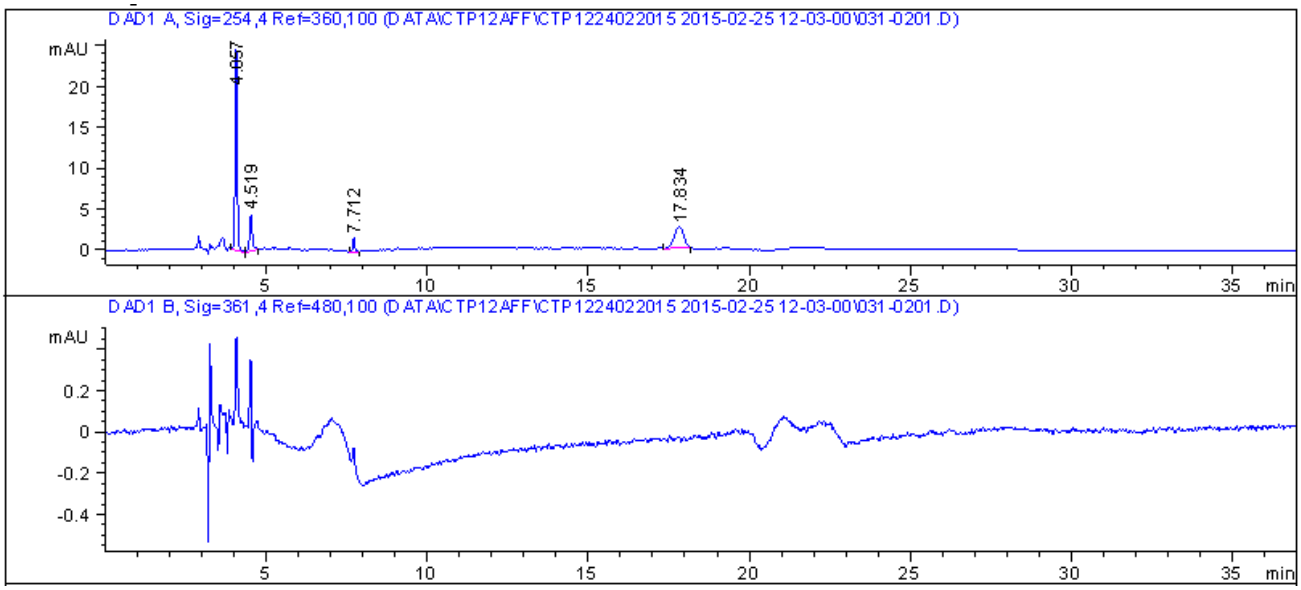


Fig. 3. Representative chromatogram for Placebo (excipients reconstituted mixture) at 254 nm and 361 nm

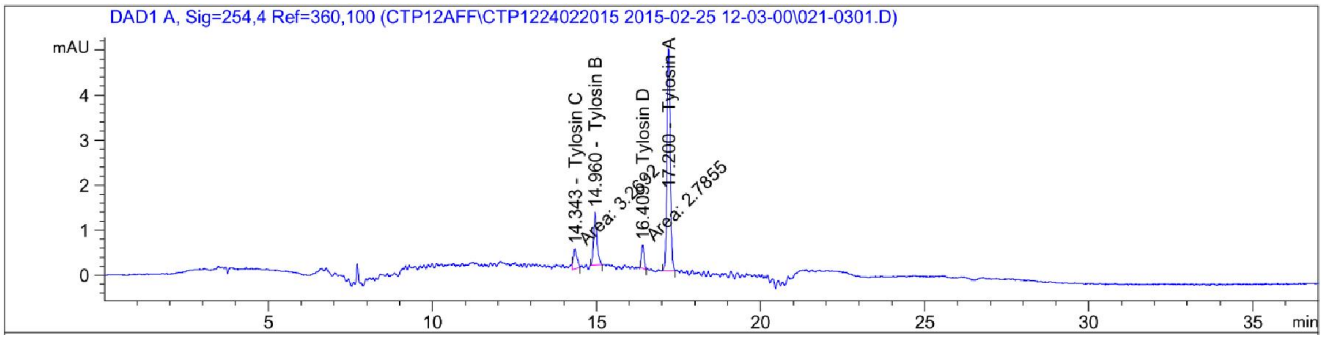


Fig. 4 . Representative chromatogram for Tylosintartrate (in elution order Tylosin A, Tylosin B, Tylosin C, Tylosin D) at 254 nm (4µL)

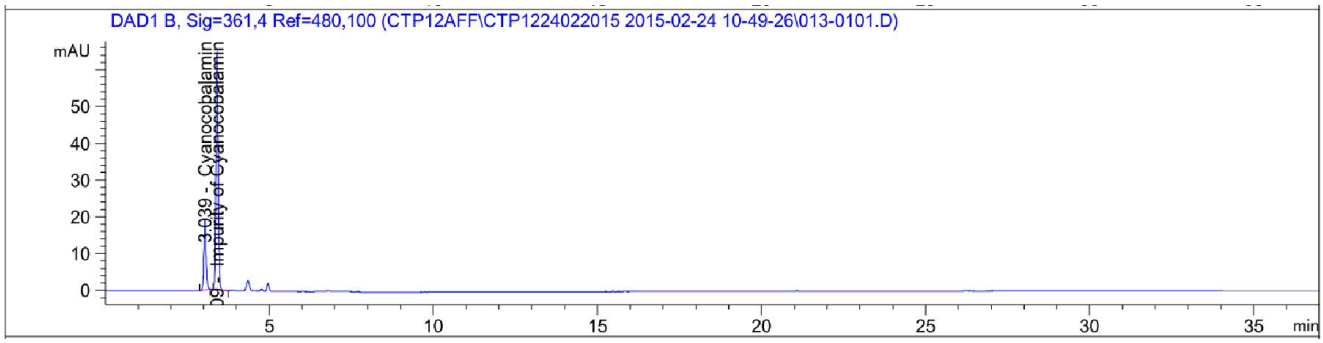


Fig. 5. Representative chromatogram for Cyanocobalamine 361 nm (see European Pharmacopoeiamonography of cyanocobalamine)

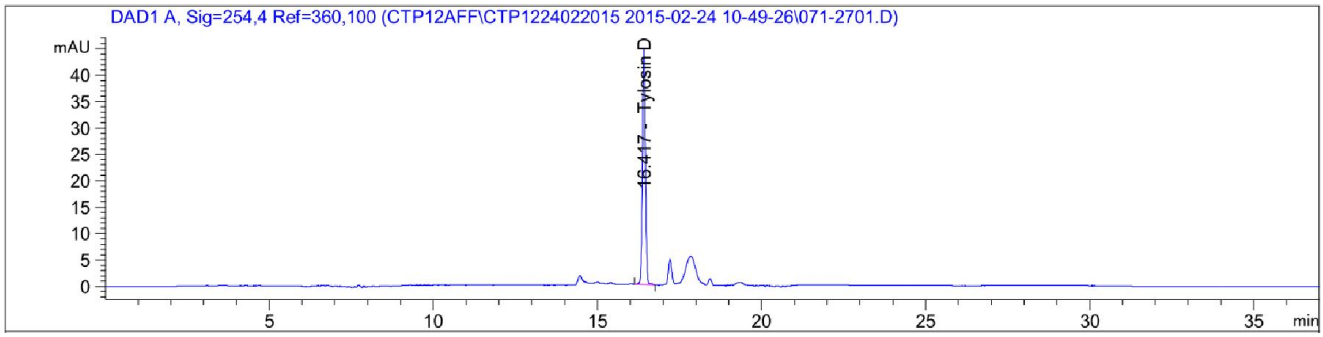


Fig. 6. Representative chromatogram for Tylosin D CRS, 254 nm (4µL)

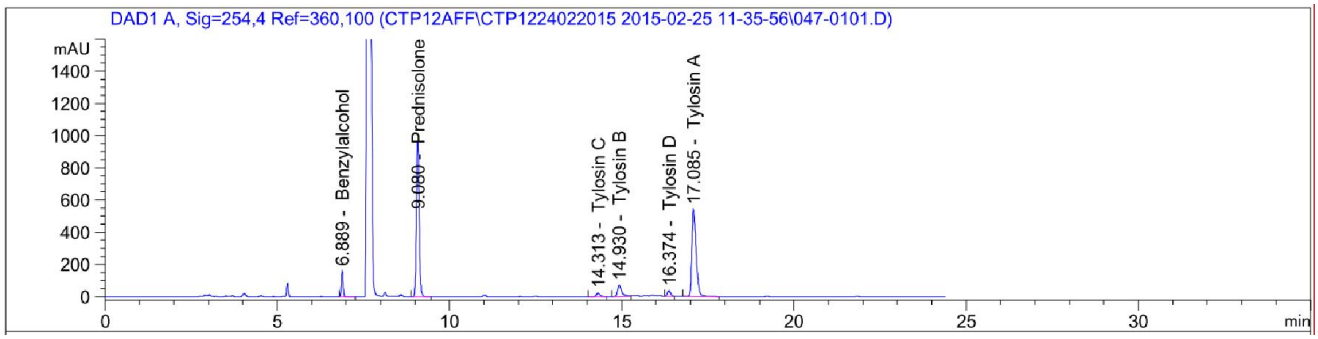


Fig. 7. Representative chromatogram for Assay reference solution at 254 nm

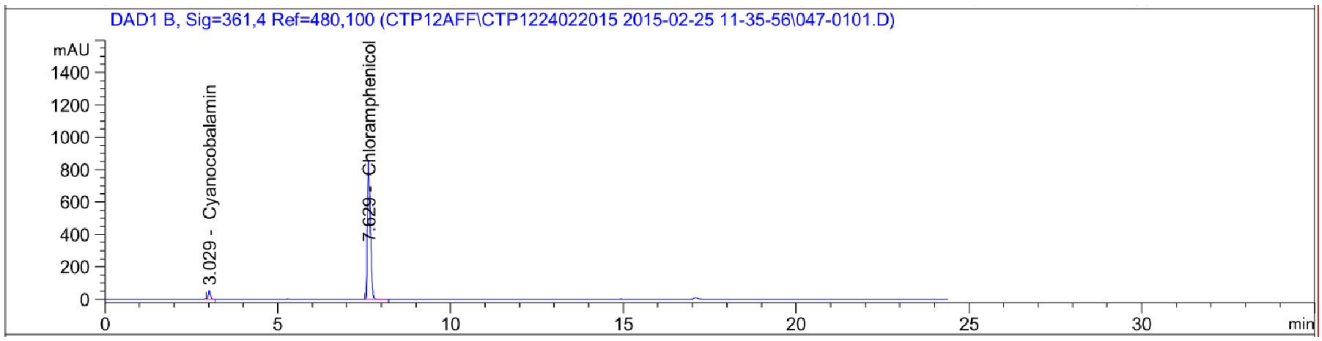


Fig. 8. Representative chromatogram for Assay reference solutionat 361 nm

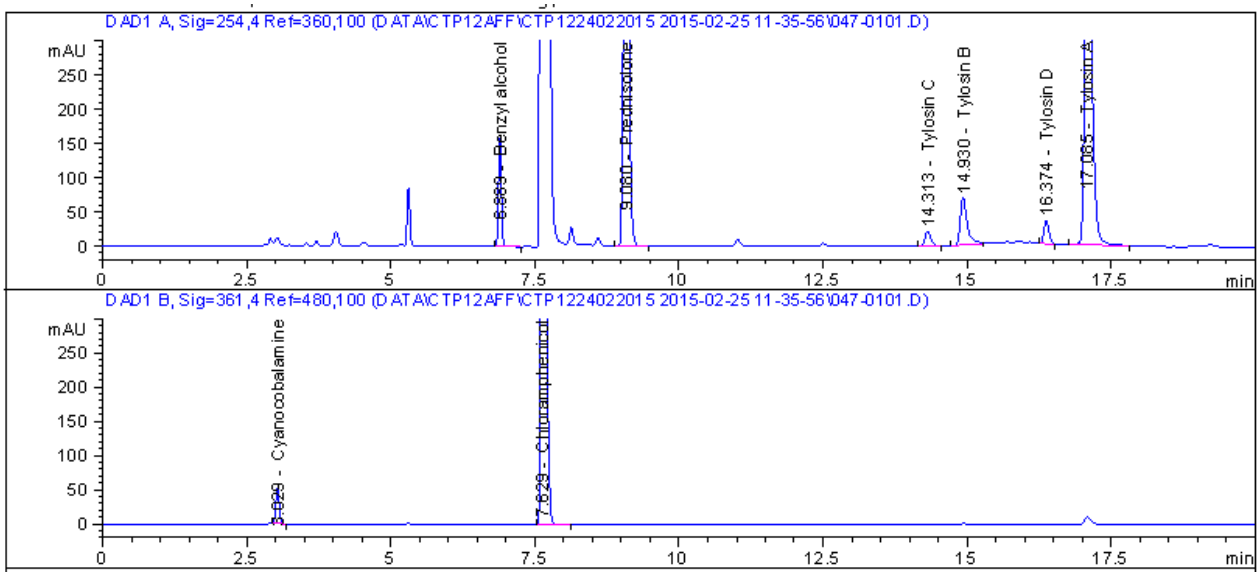


Fig. 9. Representative chromatogramatogram for Sample test (assay and related substaces sample) at λ=254 mn and repectively λ=361 nm

Limit of Detection (LOD)

According to ICH guidelines, the LOD could be determinate based on Signal-to-noise ratio. A signal to noise ratio between 3 or 2:1 is generally considerate acceptable for estimation the detection limit.

The LOD was determinate based on the signal-to-noise

Limit of Quantification (LOQ)

The Limit of Quantification is the lowest concentration of an analyte in a sample that can be determined and quantified with acceptable precision and accuracy under the stated operational conditions of the method. According to ICH guidelines, the LOQ could be determinate based on Signal-to-noise. A signal to noise ratio between 10:1 is generally considerate acceptable for estimation the quantification limit

The LOQ was determinate based on the signal-to-noise ratio (using calculation on linearity solution).

The statistical formulae in the data processing for linear regression are:

Covariance:

$$Sxy = \frac{\sum xy - \frac{\sum x - \sum y}{n}}{n-1};$$

Standard deviation for x value population:

$$Sx = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}};$$

Standard deviation for y value population:

$$Sy = \sqrt{\frac{\sum y^2 - \frac{(\sum y)^2}{n}}{n-1}};$$

Correlation coefficient:

$$rxy = \frac{S_{xy}}{S_x\ S_y};$$

Slope:

$$B = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}};$$

Intercept:

$$A = \frac{\sum y}{n} - B\ \frac{\sum x}{n};$$

Standard deviation for the whole value population:

$$S0 = \sqrt{\frac{\sum y^2 - A \sum y - B \sum xy}{n-2}}$$

Standard deviation associated with the slope:

$$Sb = \sqrt{\frac{nS_0^2}{n \sum x^2 - (\sum x)^2}}$$

Standard deviation associated with the intercept:

$$S\sigma = \sqrt{\frac{S_b^2}{n}} \sum x^2;$$

Variation range for A:

A±t*Sa;

Variation range for B:

B±t*Sb;

Identification limit:

$$Xi = \frac{2t(S_a + \frac{\sum x}{n} * S_b)}{b + 2t * S_b};$$

Where:

x = the nominal concentration value of the target analyte (µg/mL or mg/ml);

y = the mean peak area value for the target analyte, integrated in the recorded chromatograms of consecutively injections of each Linearity solutions

t = the "Student" coefficient (for a known level of certainty P% and for a number ν of freedom degrees), ν = the number of freedom degrees (ν = n-2), n = the number of sets pairs of experimental data (concentration – mean area).

RESULTS AND DISCUSSION

In this chromatographic conditions no interference peaks were found which could affect the separation of active pharmaceutical ingredients and related substance/degradation products, result that the method selectivity/specificity has been demonstrated. Method is enough sensitive to quantified and monitorised related substanes/degradation product according to VICH GL11. Range of linearity for method: For all eight active pharmaceutical ingredients $R^2 = 0.999$ on range LOQ 80÷ 120%; Precision: %RSD of Recovery = NMT 2; Accuracy: %, Recovery= 95.0 -105.0; Was verified robustness of method for all chromatografic parameters variation according to method 2.2.46 Chromatographic separation technique – European Pharmacopoeia, and result that themethod is robuste.

REFERENCES

"Chloramphenicol and Congeners". Merck Manuals. Retrieved 31 October 2014.
^ Jump up to:[a b c d] Tylosin, marvistavet.com
^ Jump up to:[a b] Tylan 200 Injectable - Product data sheet PA9063DENZP - Elanco Animal Health
Antimicrobial Therapy in Veterinary Medicine, by Steeve Giguere, P. M. Dowling, ISBN 978-0-8138-0656-3
CPMP/ICH/381/-ICH Q (R1)
European Pharmacopoeia, ed.8.0, (monographies of all eight API's and method 2.2.46 Chromatographic tecniques separation.
Falagas, M. E.; Grammatikos, A. P.; Michalopoulos, A. (October 2008). "Potential of old-generation antibiotics to address current need for new antibiotics". *Expert Review of Anti Infective Therapy* 6 (5): 593–600. doi:10.1586/ 14787210.6.5. 593. PMID 18847400.
http://antibiotics.toku-e.com/antimicrobial_507.html
ICH-Q2A –Text on Validation of Analytical Procedure
ICH-Q2B- Validation of Analytical Procedure: Methodology
Raymond C Rowe, Paul J Sheskey and Marian Quinn – Handbook of Pharmaceutical Excipients, Sixth Edition, PhP, pg. 64.
United Stated Pharmacopoeia
VICH GL1
VICH GL11 (R)
VICH GL2
www.vetinfo.com/tylosin-tartrate-for-dogs.html
Yamada, Kazuhiro 2013. "Chapter 9. Cobalt: Its Role in Health and Disease". In Astrid Sigel, Helmut Sigel and Roland K. O. Sigel. *Interrelations between Essential Metal Ions and Human Diseases*. Metal Ions in Life Sciences 13. *Springer*. pp.295–320.

****