



Received on 20 June, 2014; received in revised form, 24 September, 2014; accepted, 16 November, 2014; published 01 March, 2015

SIMULTANEOUS ESTIMATION OF SULFAQUINOXALINE SODIUM AND AMPROLIUM HYDROCHLORIDE BY RP- HPLC.

Abhilekha P. Mantri*, Rubeena Mubeen S., Nischal Kandepu and Shiva Kumar

Provimi Animal Nutrition India Pvt Ltd, C7/22, KSSIDC Industrial Area, Yelahanka Newtown, Bangalore- 560 064, Karnataka, India

Keywords:

HPLC method,
Sulfaquinoxaline
Powder, Amprolium

Correspondence to Author:

Abhilekha P. Mantri

Provimi Animal Nutrition India Pvt Ltd, C7/22, KSSIDC Industrial Area, Yelahanka Newtown, Bangalore- 560 064, Karnataka, India,


E-mail: abhilekhamantri@provimi.in

ABSTRACT: A new RP-HPLC method was developed and validated for the simultaneous determination of amprolium hydrochloride and sulfaquinoxaline sodium in a powder dosage form. The chromatographic separation was achieved by isocratic mode with Phosphate buffer and Acetonitrile (30:70; v/v), as mobile phase and using C-18 RP-Amide (25.0cm × 4.6 mm i.d, 5μ particle size) as stationary phase, with a flow rate of 1.0 ml/min at ambient temperature. The quantification of the analytes was monitored by UV detection at a wavelength of 263 nm. The optimized method was subjected to validation for specificity, accuracy, precision, linearity, range, ruggedness and robustness. The calibration graphs were linear in the concentration range of 0.08 – 0.12 mg/ml. The results were evaluated as per the International Conference on Harmonization (ICH) guidelines. The precision of the method in terms of RSD is not more than 2% for both the analytes. The method is sensitive, specific, fast and accurate with minimum sample preparation.

INTRODUCTION: Amprolium hydrochloride, 1-[(4 - amino - 2 - propyl - 5 - pyrimidinyl) methyl] - 2 -methylpyridiniumchloride, is an antiprotozoal agent used in veterinary practice for the control of coccidiosis in poultry ^{1, 2}. Sulfaquinoxaline, 4-amino-N-quinoxalin-2-ylbenzenesulfonamide, is a sulfonamide with high anticoccidial potential ³. A combination of both is used in the poultry industry to effectively combat coccidiosis. Several methods have been published for determination of Amprolium hydrochloride and Sulfaquinoxaline sodium separately using HPLC ^{4, 5, 6, 7}, TLC ⁸, ion pair chromatography ⁹ and Spectrophotometry ¹⁰.

Also simultaneous estimations using derivatization ¹¹, ion pair chromatography and using HILIC ¹² chromatography have been reported. Since both the compounds are highly polar, it is challenging to achieve desired resolution between the peaks.

It was realized that there is no simple and convenient analytical method for regular quality control checks for this powder dosage form. In the present study, a simple, rapid, and accurate method for quantitative estimation of amprolium hydrochloride and sulfaquinoxaline sodium, which are the major components of the formulation in powder dosage forms meant for poultry birds was developed using High Performance Liquid Chromatography. The method was validated with respect to linearity, precision, accuracy and robustness as per the International Conference on Harmonization (ICH) guidelines ¹³.

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.6(3).1097-00</p> <hr/> <p>Article can be accessed online on: www.ijpsr.com</p>
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(3).1097-00</p>	

MATERIALS AND METHODS:

Chemicals and reagents:

Amprolium Hydrochloride and Sulfaquinoxaline sodium standards from sigma were used. Acetonitrile (HPLC grade), and Deionized water were purified using Milli Q water system. All other reagents and solvents used in study were of analytical grade.

Equipment:

A Shimadzu HPLC system equipped with a quaternary pump, auto sampler, photo diode array detector was used. The system was connected with the help of LC solution software in a computer system for data collection and processing.

Chromatographic conditions:

The chromatographic column used was a C-18 RP-Amide (25.0 cm × 4.6 mm) with particle size of 5 µm. The mobile phase selected for the assay was prepared by mixing 0.05 M phosphate buffer (pH 5) and acetonitrile (ACN) (30:70; v/v), shaken well. The mobile phase was filtered using 0.45 µm microporous filter and was degassed by sonication prior to use. The standard and sample solutions were also filtered using 0.45 µm membrane filter. A wavelength of 263 nm was chosen since it was found the most appropriate for the determination of the two active ingredients simultaneously. The flow rate used was 1.0 ml/minute. The injection volume was 20 µl and the total run time was about 10 minutes.

Preparation of standard solution:**Stock solution:**

10 mg of amprolium hydrochloride standard and 10 mg of sulfaquinoxaline sodium were accurately weighed in to a 10 ml volumetric flask and dissolved in mobile phase. The solution was sonicated for 2 minutes and then made up to the mark with mobile phase.

Working dilution:

From the above stock solution, 1 ml was withdrawn into a 10 ml volumetric flask and made up to the mark with mobile phase. The solution was filtered using 0.45 µm membrane filter prior to injecting into HPLC.

Preparation of sample:

50 mg of the formulation was accurately weighed into a calibrated volumetric flask and added 9 ml of mobile phase and sonicated for 2 minutes. Finally the volume was made up to mark with mobile phase. 1 ml of this solution was diluted up to 10 ml with mobile phase. The solution was filtered using 0.45 µm membrane filter prior to injecting into HPLC.

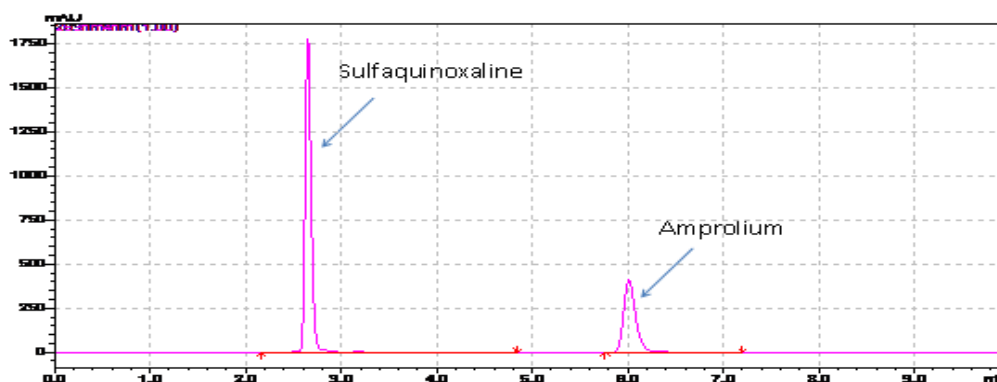
RESULTS & DISCUSSION:**Method development:**

The system suitability test was carried out by preparing a freshly prepared stock solution of amprolium hydrochloride and sulfaquinoxaline sodium to check various parameters. The system suitability results are tabulated in **Table 1**. The chromatogram of the standard is shown in **Figure 1**.

TABLE 1: SYSTEM SUITABILITY (N=6)

Parameters	Retention time(RSD%)	Theoretical plate	Tailing factor	Calibration range
Sulfaquinoxalinesodium	0.124	6657	1.38	0.08 mg/ml – 0.12 mg/ml
Amprolium hydrochloride	0.163	10007	1.36	0.08 mg/ml – 0.12 mg/ml

N: Number of repetitions

**FIGURE 1: CHROMATOGRAM OF THE STANDARD SOLUTION OF AMPROLIUM HYDROCHLORIDE AND SULFAQUINOXALINE SODIUM**

Method Validation:**Specificity:**

The placebo excluding both Amprolium hydrochloride and Sulfaquinoxaline sodium was prepared and injected. No peaks were observed in the placebo chromatograms corresponding to Amprolium hydrochloride and Sulfaquinoxaline sodium.

Linearity and range:

The linearity of the method was observed within the expected concentration range demonstrating its suitability for analysis. The correlation coefficient (R^2) was found to be 0.9988 and 0.9923 for Amprolium hydrochloride and Sulfaquinoxaline sodium respectively. The calibration curves for linearity data is shown in **Figure 2** and **Figure 3**.

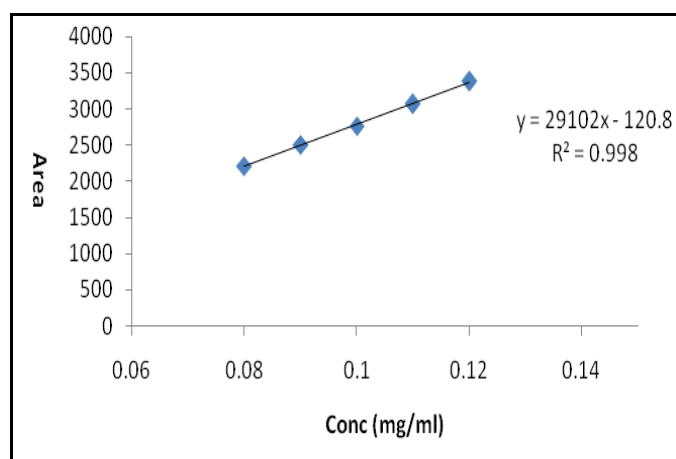


FIGURE 2: CALIBRATION CURVE FOR AMPROLIUM HYDROCHLORIDE

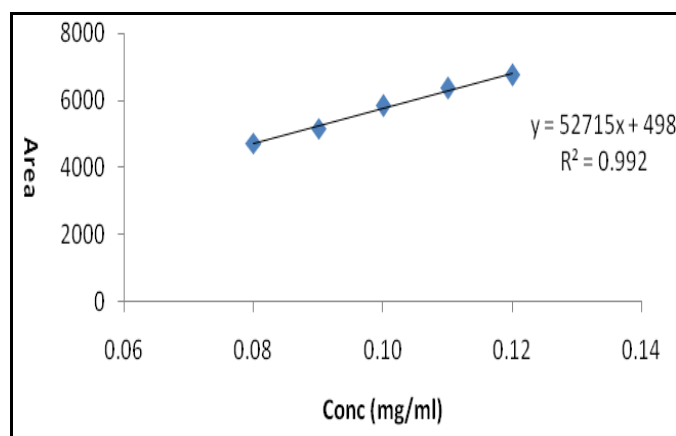


FIGURE 3: CALIBRATION CURVE FOR SULFAQUINOXALINE SODIUM

Accuracy:

The accuracy of the method was determined by addition of the active ingredients in different concentrations to the placebo matrix. The accuracy

was evaluated by the recovery of amprolium and sulfaquinoxaline at three different levels like 80, 100 & 120 % of the label claim. The mean recovery was calculated as shown in **Table 2**.

TABLE 2: RECOVERY DATA FOR THE PROPOSED METHOD

Level Recovery % (± SD) (N=3)	80 %	100 %	120%
Sulfaquinoxaline	99.57	99.34	99.81
Sodium	± 0.55	± 0.51	± 0.59
Amprolium	99.1	99.72	101.31
Hydrochloride	± 0.32	+ 0.49	± 1.04

N: Number of repetitions

SD: Standard Deviation

Precision:

Repeatability: Study was assessed by injection repeatability. The injection repeatability was confirmed by performing replicate injections of sample solution at 100% concentration and calculating % RSD of the peak area response. The data showed good precision of the system with the RSD <2%.

Intermediate precision: Intermediate precision was performed by 2 analysts on three replicates of each sample at different points of time. The RSD was found to be < 2%.

Reproducibility: The ruggedness study was carried out for sulfaquinoxaline sodium and amprolium hydrochloride on the powder dosage formulation at 100% level of the final test concentration with different analytical equipment's on different days. The RSD percentage values showed that the results of the assay of the two active ingredients are within a suitable range of < 2%.

Robustness:

The robustness of analytical method was studied by analyzing the samples with slight variation in the mobile phase composition, Condition-1 {Buffer: Acetonitrile (69: 31)} and {Buffer: Acetonitrile (71:29)}; and also by analyzing the samples with slight variation in the pH of the buffer (4.9 and 5.1), Condition - 2. The RSD was found to be < 2%. The statistical evaluation of the two active ingredients for repeatability, Intermediate precision, reproducibility and robustness are tabulated in **Table 3**.

TABLE 3: THE RSD VALUES OF REPEATABILITY, INTERMEDIATE PRECISION, REPRODUCIBILITY AND ROBUSTNESS

Parameters	Amprolium Hydrochloride	Sulfaquinoxaline Sodium
RSD, % (Repeatability) (N=6)	1.107	0.856
RSD, % (Intermediate Precision)	0.385	0.51
RSD, % (Reproducibility) (N=3)	0.93	0.59
RSD, % (Robustness) Condition -1	0.744	0.538
RSD, % (Robustness) Condition -2	0.79	0.738

N: Number of repetitions

CONCLUSION: The proposed method is simple, sensitive, precise and accurate. There are no additional extraction or separation procedures to extract the actives from the formulation matrix thereby decreasing the error in quantitation. Also the method is convenient for routine use and is less time consuming. Therefore this method can be applied for routine quality control analysis of a combination of Amprolium hydrochloride and Sulfaquinoxaline sodium in powder dosage forms.

ACKNOWLEDGEMENT: We copiously thank the management of Provimi Animal Nutrition India Pvt Ltd., Bangalore for the support provided throughout the study.

REFERENCES:

1. Cynthia M. Kahn and Scott Line: The Merck Veterinary Manual. Merck & Co, Whitehouse Station, N.J, Edition 10, 2011.
2. British Pharmacopoeia Veterinary. Amprolium Hydrochloride Monograph. London. 2013: 42-44.
3. William C Campbell: History of the Discovery of Sulfaquinoxaline as a Coccidiostat. Journal of Parasitology 2008; 94(4): 934-945.
4. The United States Pharmacopeia national formulary, United State Pharmacopeial Convention Inc., Amprolium soluble powder Monograph. 2011; 1901.
5. Lakkanatinaporn P and Matayatsuk C: Simultaneous HPLC method for determination of sodium trimethoprim phenylpropanoldisulphonate and sodium sulfaquinoxaline in veterinary drugs. Songklanakarin Journal of Science and Technology 2004; 26(6): 849-854.
6. Márcia N. S. de la Cruz, Renata F. Soares, Anna Sylvia F. Marques and Francisco Radler de Aquino-Neto: Development and Validation of Analytical Method for Sulfonamide Residues in Eggs by Liquid Chromatography Tandem Mass Spectrometry based on the Commission Decision 2002/657/EC. Journal of the Brazilian Chemical Society 2011; 22: 454-461.
7. Anna Martínez-Villalba, Encarnación Moyano and M. Teresa Galceran: Analysis of amprolium by hydrophilic interaction liquid chromatography-tandem mass spectrometry. Journal of Chromatography A2010; 1217(37): 5802-5807.
8. Nahla N. Salama, Manal M. Fouad, Noha M. Rashed: Validated chromatographic methods for simultaneous determinations of Amprolium hydrochloride and Ethopabate in veterinary preparations. International Journal of Pharmaceutical and Biomedical Research 2012; 3(4): 185-190.
9. Kentzer E. J, Cottingham L. S and Smallidge R. L: Ion-pair reverse-phase liquid chromatographic determination of amprolium. Journal - Association of Official Analytical Chemists 1988; 71(2): 251-255.
10. Yousry M. Issa, Mahmoud S. Rizk, Adel F. Shoukry and Ehab M. Atia: Extraction-Spectrophotometric Determination of Amprolium Hydrochloride using Bromocresol Green, Bromophenol Blue and Bromothymol Blue. Mikrochimica Acta 1997; 127: 269-272.
11. Takahashi Y, Sekiya T, Nishikawa M, and Endoh Y. S: Simultaneous High-Performance Liquid Chromatographic Determination of Amprolium, Ethopabate, Sulfaquinoxaline and N4-Acetylsulfaquinoxaline in Chicken Tissues. Journal of Liquid Chromatography 1994; 17(20): 4489-4512
12. Mashhour Ghanem and Saleh Abu-Lafi: Validation of a Stability-Indicating Assay of Amprolium Hydrochloride in Water Soluble Powder Formulation using Hydrophilic Interaction Liquid Chromatography. Journal of Applied Pharmaceutical Science 2013; 3(10): 051-058.
13. ICH Harmonized Tripartite Guideline, Q2 (R1), Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, Geneva.2005: 1-13.

How to cite this article:

Mantri AP, Rubeena MS, Kandepu N and Kumar S: Simultaneous Estimation of Sulfaquinoxaline Sodium and Amprolium Hydrochloride by RP- HPLC. Int J Pharm Sci Res 2015; 6(3): 1097-00.doi: 10.13040/IJPSR.0975-8232.6 (3).1097-00.

All © 2014 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)