

# Validated HPLC Method to Simultaneously Determine Amprolium Hydrochloride, Sulfaquinoxaline Sodium and Vitamin K<sub>3</sub> in A.S.K Powder on ZIC-HILIC Column

Mashhour Ghanem<sup>1</sup>, Saleh Abu-Lafi<sup>2\*</sup>, Rafik Karaman<sup>2</sup> and Hussein Hallak<sup>2</sup>

<sup>1</sup>Pharmacare Pharmaceutical Company, P.O. Box 677, Ramallah, Palestine

<sup>2</sup>Faculty of Pharmacy, Al-Quds University, P.O. Box 20002, Abu-Dies, Palestine

## Abstract

A new HPLC method that is based on zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) coupled with ultraviolet detection was developed, optimized and validated for the simultaneous determination of amprolium hydrochloride, sulfaquinoxaline sodium, and Vitamin K<sub>3</sub> (as menadione sodium bisulfite) in A.S.K powder. The separation was carried out using ZIC-HILIC column (250 mm × 4.6 mm, 5 μm) and a mobile phase of 0.2 M Ammonium acetate (NH<sub>4</sub>AC) buffer and acetonitrile (15:85; v/v) with pH adjusted to 5.7 by glacial acetic acid at a flow rate of 0.5 ml/min. The analytes were monitored by UV detection at 263 nm.

The effects of the operational chromatographic conditions on retention and resolution were tested. Different concentrations of the organic solvent in the mobile phase, the ionic strength of the NH<sub>4</sub>AC buffer and pH of the mobile phase were investigated.

The optimized method was subjected to validation by examining specificity, accuracy, precision, linearity, range, ruggedness and robustness. The results were evaluated as per the International Conference on Harmonization (ICH) and United States Pharmacopoeia (USP33/NF28) guidelines and it fulfilled the validation criteria. The method is sensitive, specific, fast, accurate, and requires minimum sample manipulation. It was applied on commercial A.S.K batches, to which all the active ingredients were separated from their excipients.

**Keywords:** Amprolium hydrochloride; Sulfaquinoxaline sodium; Vitamine K<sub>3</sub>; ZIC-HILIC; Validation

## Introduction

A.S.K Powder is a generic veterinary drug manufactured by Pharmacare pharmaceutical company (Palestine). The A.S.K powder is mainly used for the treatment and control of coccidiosis disease. It is very similar in composition to Amprocoxin-silv<sup>®</sup> that is manufactured by Silvavet Company for veterinary medicines.

The A.S.K powder comprises Amprolium hydrochloride, Sulfaquinoxaline sodium and Vitamin K<sub>3</sub> sodium bisulfite (as menadione sodium bisulfite).

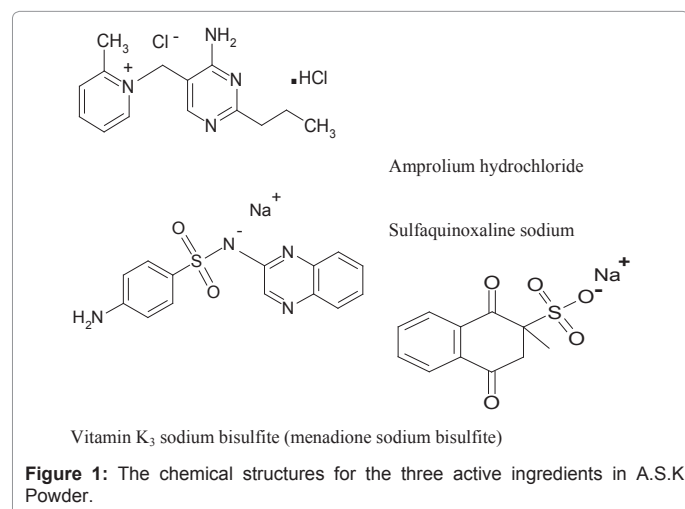
As shown in Figure 1, the three active ingredients of the A.S.K

Powder are very hydrophilic and possess polar basic or acidic character. Therefore, under most circumstances, they would elute within the column void volume, thus resulting in a significant challenge to keep them retained when using reversed phase chromatography.

In order to enhance their retention without using derivatization methodologies or adding ion-pair reagent to the mobile phase, a new HPLC method which is based on zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) coupled with ultraviolet detection has been optimized and validated.

To our knowledge, there is no quality control method neither in the official Pharmacopoeias nor in the literature, that determines the drug active ingredients simultaneously and therefore A.S.K Powder was not registered at the Ministry of Health of Palestine till now.

There are different non-chromatographic and chromatographic methods, particularly RP-HPLC and ion-pair RP-HPLC, which report about the assay of each active ingredient individually or when present in combinations with other ingredients [1-17]. Some of these



**\*Corresponding author:** Saleh Abu-Lafi, Faculty of Pharmacy, Al-Quds University, P.O. Box 20002, Abu-Dies, Palestine, Tel: + 970-2-2790413, Fax: +970-2-2790413; E-mail: [sabulafi@science.alquds.edu](mailto:sabulafi@science.alquds.edu)

**Received** July 26, 2012; **Accepted** September 18, 2012; **Published** September 25, 2012

**Citation:** Ghanem M, Abu-Lafi S, Karaman R, Hallak H (2012) Validated HPLC Method to Simultaneously Determine Amprolium Hydrochloride, Sulfaquinoxaline Sodium and Vitamin K<sub>3</sub> in A.S.K Powder on ZIC-HILIC Column. Pharmaceut Anal Acta 3:168. doi:10.4172/2153-2435.1000168

**Copyright:** © 2012 Ghanem M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

methods suffer from drawback of using ion-pair reagents which include sensitivity reduction, extended time to reach equilibration, cleaning and regeneration of the column.

Therefore, there is a need to develop a fast, specific, and accurate method that allows the simultaneous determination of the three active ingredients within a reasonable retention time as per ICH/USP validation norms [18,19] (Figure 1).

## Experimental Details

### Chemicals

Samples of amprolium hydrochloride, sulfaquinoxaline sodium, vitamin K<sub>3</sub> sodium bisulfite reference standards were purchased from Sigma-Aldrich (Germany). Ammonium acetate extra pure, acetic acid (glacial), acetonitrile (ACN) and methanol HPLC grade solvents, hexane-1-sulfonic acid sodium salt and decane-1-sulfonic acid sodium salt were purchased from Merck (Germany).

High purified water was prepared by using a Millipore Milli-Q plus water purification system.

A.S.K Powder samples, and all the active ingredients and excipients usually used in manufacturing the pharmaceutical combination, were kindly supplied by Pharmacare pharmaceutical company, Palestine.

### Equipments

Elite Lachrom high performance liquid chromatograph equipped with uv-detector and supported with autosampler and column oven and Elite Lachrom data system of Agilent (Merck Hitachi, England).

The Ultraviolet/visible spectrometer (PG Instruments, United Kingdom).

### Chromatographic conditions

The chromatographic column used was a ZIC-HILIC column (25.0 cm × 4.6 mm) with particle size of 5 μm (Merck, Germany) protected with a ZIC-HILIC guard column (20 mm × 2.1 mm, 5 μm).

0.2 M ammonium acetate solution was prepared by dissolving 3.08 g of NH<sub>4</sub>AC in purified water and diluted up to 200 ml with the same solvent.

The optimum mobile phase selected for the assay was prepared by mixing 0.2 M NH<sub>4</sub>AC and acetonitrile (ACN) (15:85; v/v), shaken well and left till the temperature of the mobile phase reached to the room temperature. Then the pH was adjusted to 5.7 by glacial acetic acid. 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 three active ingredients simultaneously. The flow rate used was 0.5 ml/minute as recommended by the column manufacturer whereby minimum height equivalent of theoretical plates and maximum number of theoretical plates are expected to be generated.

The injection volume was 20 μl and the temperature of the auto-sampler was 15°C and that of the column was 25°C. Total run time was about 18 minutes.

### Preparation of stock and standard solutions

Stock solution for menadione sodium bisulfite was prepared by

dissolving menadione sodium bisulfite reference standard equivalent to 20 mg menadione (vitamin K<sub>3</sub>) in 80.0 ml of 90% ACN and diluting up to 100.0 ml with the same solvent. 5 ml of this solution was diluted up to 50 ml with 90% ACN to yield a solution with a final concentration of 20.0 μg/ml.

Standard solutions for amprolium HCl, sulfaquinoxaline sodium and vitamin K<sub>3</sub> were prepared by dissolving 20.0 mg of amprolium hydrochloride reference standard, 20 mg sulfaquinoxaline sodium reference standard in 70.0 ml of 90% ACN, 10 ml of menadione sodium bisulfite stock solution was added, mixed well and then diluted to 100.0 ml with 90% ACN. 5 ml of this solution was diluted up to 50 ml with mobile phase. The solution was filtered using 0.45 μm membrane filter. The obtained final solution contains 20 μg/ml for amprolium hydrochloride, 20 μg/ml sulfaquinoxaline sodium and 0.20 μg/ml vitamin K<sub>3</sub>. This solution has been used within 24 hours and kept at 15°C and was protected from light.

### Preparation of sample solution

Sample solution was prepared by dissolving 100 mg of A.S.K Powder in 80 ml of 90% ACN and then diluted up to 100 ml with the same solvent. Then 5 ml of the solution was diluted up to 50 ml with mobile phase. The latter solution was filtered using 0.45 μm membrane filter. The obtained final solution contains 20 μg/ml for amprolium hydrochloride, 20 μg/ml sulfaquinoxaline sodium and 0.20 μg/ml vitamin K<sub>3</sub>. This solution has been used within 24 hours and kept at 15°C and was protected from light.

## Results and Discussion

### Development and optimization of the ZIC-HILIC-HPLC method

Since the combined active ingredients are highly hydrophilic, at first we have tried the ion pair RP-chromatography mode using sodium 1-hexanesulfonate and decane-1-sulfonic acid sodium salts at different concentration levels. Poor peak profile shapes with small resolution (Rs) values were obtained. Moreover, the ion pair reagents decreased the assay sensitivity for all ingredients particularly vitamin K<sub>3</sub> since it is present at 0.2% level of the overall mixture concentration. This unsatisfactory start, lead us to switch to ZIC-HILIC column which is designed to overcome the shortcomings of the ion-pair mode when separating very polar compounds in reversed phase chromatography.

The overlaid ultraviolet absorption spectra of the three active ingredients demonstrated that they shared a wavelength near to 263 nm, and therefore it was chosen during the entire study.

Different mobile phases have been employed in order to optimize the desired HPLC method using HILIC column. These mobile phases differ in the concentration of NH<sub>4</sub>AC buffer, pH, and organic solvent strength.

**The effect of NH<sub>4</sub>AC buffer concentration:** Our first choice of a mobile phase was ACN/10 mM NH<sub>4</sub>AC buffer solution (80:20; v/v). The pH of the mobile phase was adjusted to 5.0. The ionic strength of the mobile phase started at 10 mM and was increased up to 200 mM (Figure 1). As the ionic strength of the mobile phase increased, the analysis time decreased and the resolution (Rs) increased among all separated drugs. When the concentration of NH<sub>4</sub>AC was at 20 mM, peaks overlapped with no baseline separation between sulfaquinoxaline

sodium and vitamin K<sub>3</sub> ingredients. The amprolium hydrochloride eluted far away with a total analysis time of about 30 minutes, as shown in Figure 2.

At 75 mM, a new tiny peak was observed between sulfaquinoxaline sodium and vitamin K<sub>3</sub>, which is due to a sulfaquinoxaline related impurity (Compound A) [4]. The best separation was achieved at mobile phase ionic strength of 200 mM. A resolution of >2.5 was achieved between sulfaquinoxaline sodium, its impurity and the other active ingredients with a total analysis time of 16 minutes. The achieved shorter retention time implies that the interaction is based on hydrophilic nature as the ionic strength increased. The positively charged ammonium ions in the mobile phase may weaken the hydrophilic interaction between the protonated ingredients and the stationary phase which result in shortening the retention time.

**The effect of the mobile phase pH:** A study of the pH effect on the resolution was deemed necessary to further optimize the separation conditions. The resolution values for all the ingredients under investigation were significantly influenced by the variation of the mobile phase pH. The pH range tested was from 3.5 up to 7.0. The best result was found at a pH between 5.6 and 5.8 (Figure 3). Maximum resolution for all ingredients was accomplished at pH 5.7.

As the pH increased, the retention time of all the ingredients slightly increased. The electrostatic interactions between the positively charged basic ingredients and the negatively charged surface silanol groups increased resulting in an increase of the retention time of all active ingredients. Final pH selected for the mobile phase was pH 5.7.

**The effect of acetonitrile percentage:** The effect of acetonitrile organic solvent percentages at fixed NH<sub>4</sub>AC buffer concentration and mobile phase pH of 5.7 on retention and resolution was also investigated. It was found that increasing the ratio of ACN in the mobile phase resulted in an increase in the retention time and improved the peaks resolution. The best separation was achieved when 85% ACN was used (Figure 4).

By increasing the percentage of ACN, the polarity of the mobile phase decreased and the hydrophilic interactions between the analytes and the stationary were promoted, thereby dramatically increasing the retention of the ingredients.

The effect of acetonitrile on the retention time was systematically increased up to 90% ACN. However, when an extra 5% of ACN was added, the peak of vitamin K<sub>3</sub> eluted in a close proximity to amprolium HCl. This probably indicates a sudden disruption in the equilibrium coefficient of this compound in the HILIC column. As such ACN of 85% was selected for the mobile phase.

The effect of using methanol in addition to ACN as organic solvent modifier proved no significant effects on retention time and peaks resolution.

### The effect of temperature

The effect of a column temperature on peak resolution and tailing factor was another chromatographic parameter that was studied during the optimization of HPLC methods. Different temperatures such as 15°C, 20°C, 25°C, 30°C, were evaluated. It was found that varying temperatures between 15°C and 30°C had subtle influence on R<sub>s</sub> values. The best tailing factor obtained was at temperatures of about 25°C and 30°C.

From all the accumulated results, the optimized mobile phase consists of a mixture of 0.2M NH<sub>4</sub>AC solution and ACN organic solvent (15:85; v/v); at pH 5.7 at room temperature. During the method development process, the concentrations of the ingredients prepared were 20 µg/ml of sulfaquinoxaline sodium, 10 µg/ml of vitamin K<sub>3</sub>, and 20 µg/ml of amprolium hydrochloride. Figure 5 shows typical chromatograms obtained for the combined standard mixture using the optimized conditions.

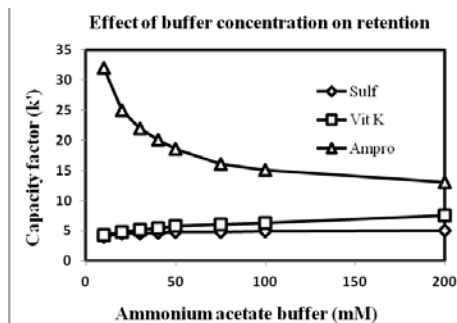


Figure 2: Effect of ionic strength on the retention of sulfaquinoxaline sodium (Sulf) (20 µg/ml), vitamin K<sub>3</sub> (Vit K) (10 µg/ml) and amprolium hydrochloride (Ampro) (20 µg/ml). Mobile phase consisting of ACN and NH<sub>4</sub>AC solution (80:20; v/v) adjusted to pH of 5.0; flow rate=0.5 ml/min; λ=263 nm; the concentration of NH<sub>4</sub>AC is as shown in the figure.

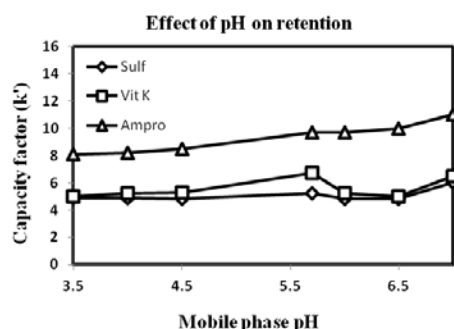


Figure 3: Effect of mobile phase pH on the retention of sulfaquinoxaline sodium (Sulf) (20 µg/ml), vitamin K<sub>3</sub> (Vit K) (10 µg/ml) and amprolium hydrochloride (Ampro) (20 µg/ml). Mobile phase consisting of ACN and 0.2M NH<sub>4</sub>AC solution (80:20; v/v); flow rate=0.5 ml/min; λ=263 nm; the pH of the mobile phase is as shown in the figure.

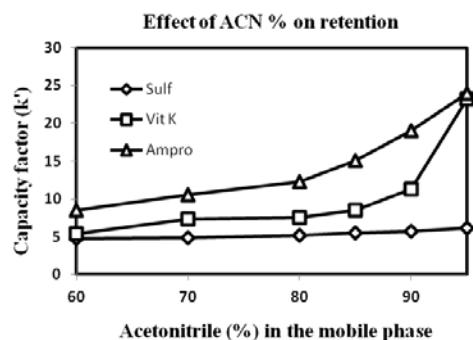
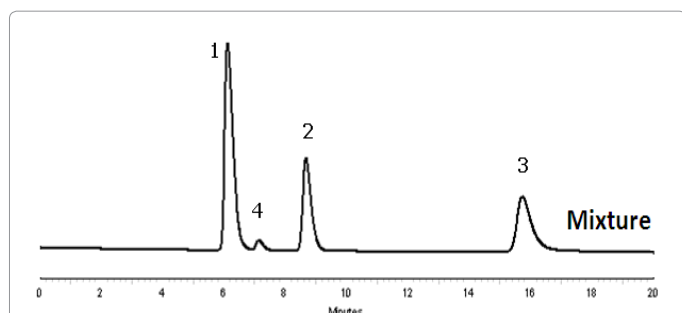


Figure 4: Effect of ACN % on the retention of sulfaquinoxaline sodium (Sulf) (20 µg/ml), vitamin K<sub>3</sub> (Vit K) (10 µg/ml) and amprolium hydrochloride (Ampro) (20 µg/ml). Mobile phase consisting of ACN and 0.2M NH<sub>4</sub>AC solution adjusted to pH of 5.7; flow rate=0.5 ml/min; λ=263 nm; the ACN% is as shown in the figure.



**Figure 5:** Typical chromatogram of standard mixture of sulfaquinoxaline sodium (1), vitamin K<sub>3</sub> (2) and amprolium hydrochloride (3); Column, a ZIC-HILIC column (25.0 cm long × 4.6 mm i.d.) with particle size of 5 µm protected with a ZIC-HILIC guard column (20 mm × 2.1 mm, 5 µm); Mobile phase consisting of ACN and 0.2M NH<sub>4</sub>AC solution (85:15; v/v) at pH 5.7; flow rate 0.5 ml/min; λ at 263 nm; column temperature of 25°C; Autosampler temperature of 15°C. Peak 4 is due to sulfaquinoxaline impurity A.

## Method validation

The validation was carried out according to the ICH/USP guidelines [18,19]. Parameters such as specificity; linearity; range; accuracy (recovery); precision (repeatability and intermediate precision); robustness and stress test were all validated.

**Specificity (placebo interference):** The chromatograms of the placebo solution, standard and test solutions were recorded at the same wavelength in order to check the specificity of the method. No peaks were observed when the placebo, dextrose monohydrate, that lacks any chromophore was injected.

The retention time of the sulfaquinoxaline sodium, amprolium hydrochloride, and menadione sample peaks match exactly the peaks of the standard solution. No peaks were present at these retention times in the placebo chromatogram. Therefore, this method is suitable for the identification and quantification of the active ingredients in the A.S.K powder.

**Linearity and range:** Different amounts of sulfaquinoxaline sodium, amprolium hydrochloride and menadione (as sodium bisulfite) in the range of 60% to 130% of the labeled amount (5 concentration levels/3 replicates each) were added to A.S.K matrix (dextrose monohydrate).

The linearity for sulfaquinoxaline sodium and amprolium hydrochloride in the range of 12 µg/ml to 26 µg/ml was investigated. The regression lines demonstrated linearity in the tested range. The regression analysis confirmed that the deviation of the y-intercept from zero is not significant; and the regression lines were linear with (*r*) of 0.9997 for sulfaquinoxaline sodium and (*r*) of 0.9998 for amprolium hydrochloride.

As to vitamin K<sub>3</sub>, the linearity was tested in a much smaller concentration within the range of 0.12 µg/ml to 0.26 µg/ml. The regression line was linear with (*r*) of 0.9983 (Table 1).

**Accuracy (recovery):** The different concentrations of the ingredients mixture were added to placebo matrix and the accuracy was measured as reflected by recovery. The data obtained for the evaluation of the linearity were used. The accuracy as reflected from recovery data and statistical evaluation for assay of the three active ingredients is listed in table 2. The recovery data of sulfaquinoxaline sodium, amprolium hydrochloride and vitamin K<sub>3</sub> showed results between 97.8% and

102% with RSD of less than 2.0% and therefore the acceptance criteria were fulfilled.

## Precision

**Repeatability:** One laboratory analyst carried out the assay of sulfaquinoxaline sodium, amprolium hydrochloride and vitamin K<sub>3</sub> on six determinations of homogeneous sample of A.S.K powder at 100% level of the test concentration with the same analytical equipment at the same day. The repeatability results of the peak areas and statistical evaluation for assay of the three active ingredients are listed in table 3.

**Intermediate precision (ruggedness):** Two laboratory analysts carried out the assay of sulfaquinoxaline sodium, amprolium hydrochloride and vitamin K<sub>3</sub> on twelve homogeneous samples of A.S.K powder at 100% level of the final test concentration with different analytical equipments at different days. The assay results and statistical evaluation for assay of the three active ingredients is listed in table 3.

The RSD percentage values showed that the results of the assay of the three active ingredients are within a suitable intermediate precision for the specified range.

**Robustness:** Robustness of the investigated HILIC-HPLC new method included five deliberate variations to some chromatographic parameters as summarized in Table 4. The modifications include different mobile phase flow rates of 0.45, 0.50, and 0.55 ml/min and three different column temperatures in the range 23-27°C. Ammonium acetate buffer in the range of (±3 of the nominal value) were also investigated. Three column batches filled with the prescribed stationary phases were studied. Finally three different pH values of the mobile

Active ingredient	Regression equation	Correlation coefficient (R)	Linear range (µg/ml) 60% to 130% of labeled amount
Amprolium HCl	y = 269332x + 62202	0.9998	(12-26)
Sulfaquinoxaline sodium	y = 597297x - 11291	0.9997	(12-26)
Vitamin K <sub>3</sub>	y = 555682x - 644.34	0.9983	(0.12-0.26)

**Table 1:** Linear ranges of the three active ingredients: amprolium HCl, sulfaquinoxaline sodium and vitamin K<sub>3</sub> (menadione sodium bisulfite).

Active ingredient	Amount added (level %)	Average recovery (%)	RSD (%)
Sulfaquinoxaline sodium	12 µg/ml (60%)	99.2	0.25
	16 µg/ml (80%)	99.7	0.49
	20 µg/ml (100%)	98.3	0.21
	24 µg/ml (120%)	99.2	0.35
	26 µg/ml (130%)	99.5	0.06
Amprolium HCl	12 µg/ml (60%)	98.9	0.35
	16 µg/ml (80%)	98.7	0.10
	20 µg/ml (100%)	99.7	0.12
	24 µg/ml (120%)	98.2	0.12
	26 µg/ml (130%)	98.1	0.06
Vitamin K <sub>3</sub>	0.12 µg/ml (60%)	102.0	0.21
	0.16 µg/ml (80%)	99.5	0.06
	0.20 µg/ml (100%)	97.8	0.68
	0.24 µg/ml (120%)	100.5	0.57
	0.26 µg/ml (130%)	101.5	0.32

**Table 2:** Average recoveries, RSD values at five concentration levels of spiking (n=3).

Active ingredient	Repeatability RSD% (n=6)	Intermediate Precision RSD% (n=6)
Sulfaquinoxaline Sodium	0.77	0.66
Amprolium HCl	0.65	0.59
Vitamin K <sub>3</sub>	0.82	0.90

**Table 3:** Repeatability and the intermediate precision of the three active ingredients.

Active ingredients	parameter	Average assay (%) (n=3)	RSD% (n=3)
Sulfaquinoxaline Sodium	Flow rate (ml/min)	100.9	0.63
	Temperature (°C)	100.5	1.02
	[NH <sub>4</sub> AC]	100.8	1.26
	Column batches (Lots)	101.6	0.12
	Mobile phase pH	101.3	0.67
Amprolium HCl	Flow rate (ml/min)	101.0	0.49
	Temperature (°C)	100.8	0.80
	[NH <sub>4</sub> AC]	101.0	1.02
	Column batches (Lots)	101.4	0.14
	Mobile phase pH	101.0	0.47
Vitamin K <sub>3</sub>	Flow rate (ml/min)	100.4	0.90
	Temperature (°C)	100.6	0.65
	[NH <sub>4</sub> AC]	101.8	0.69
	Column batches (Lots)	102.0	0.90
	Mobile phase pH	100.9	0.65

**Table 4:** Robustness testing of the three active ingredients.

phase at 5.6, 5.7, and 5.8 were tested. The RSD percentage values showed no significant change in the final assay results of each of the above three active ingredients using the five variations.

## Conclusion

The HPLC method we have developed for quantitative determination of sulfaquinoxaline sodium, vitamin K<sub>3</sub>, sodium bisulfite, and amprolium hydrochloride in A.S.K powder was evaluated over the linearity, precision, accuracy, specificity, ruggedness and robustness. All the assay validation results were within the allowed specifications of ICH/USP guidelines. The developed method is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for the simultaneous determination of the combined drugs in A.S.K formulation.

In summary, the proposed method can be used for the drug analysis in routine quality control of A.S.K formulation.

## Acknowledgments

We would like to thank Pharmicare pharmaceutical company (Palestine) for their support of equipments, materials, columns needed to finish this study.

## References

1. British Pharmacopoeia (2007) Amprolium hydrochloride Monograph.
2. United States Pharmacopeia 33–NF28 (2010) Amprolium Monograph.
3. British Pharmacopoeia (2007) Sulfaquinoxaline Monograph.
4. United States Pharmacopeia 33–NF28 (2010) Sulfaquinoxaline Monograph.

5. United States Pharmacopeia 33–NF28 (2010) Menadione monograph.
6. United States Pharmacopeia 33–NF28 (2010) Menadione injection monograph.
7. British Pharmacopoeia (2007) Menadione Monograph.
8. Chenga TC, Huang YT, Chang CY, Yao KS, Hwang CC (2009) Molecular recognition of sulfaquinoxaline and sulfapyridine with molecularly imprinted polymer. *Journal of the Chilean Chemical Society* 54: 295-298.
9. Lakkannatnarn P, Matayatsuk C (2004) Simultaneous HPLC method for determination of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline in veterinary drugs. *Songklanakarin Journal of Science and Technology* 26: 849-854.
10. de la Cruz MNS, Soares RF, Marques ASF, de Aquino-Neto FR (2011) 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. *J Braz Chem Soc* 22: 454-461.
11. Furusawa N, Kishida K (2001) High-performance liquid chromatographic procedure for routine residue monitoring of seven sulfonamides in milk. *Fresenius J Anal Chem* 371: 1031-1033.
12. Kao YM, Chang MH, Cheng CC, Chou SS (2001) Multiresidue Determination of Veterinary Drugs in Chicken and Swine Muscles by High Performance Liquid Chromatography. *Journal of Food and Drug Analysis*, 9: 84-95.
13. Filimonov VN, Zamuraev OV, Balyatinskaya LN, Kolosova IF (2000) Selection of a Ternary Eluant for the Determination of Fat-Soluble Vitamins in Multivitamin Pharmaceutical Preparations by High-Performance Liquid Chromatography. *Journal of Analytical Chemistry* 55: 657-662.
14. Boulaire SL, Bauduret JC, Andre F (1997) Veterinary Drug Residues Survey in Meat: An HPLC Method with a Matrix Solid Phase Dispersion Extraction. *J. Agric Food Chem* 45: 2134–2142.
15. Tan HS, Ramachandran P, Cacini W (1996) High performance liquid chromatographic assay of amprolium and ethopabate in chicken feed using solid-phase extraction. *J Pharm Biomed Anal* 15: 259-65.
16. Hindle R (2003) A validated atmospheric pressure chemical ionization method for analyzing sulfonamides in pork muscle. Agilent Technologies Inc. Application 1-14.
17. United States Pharmacopeia 33–NF28 (2010): Validation of Compendial procedures.
18. ICH, Q2 (R1) (2005) Validation of Analytical Procedures: Text and Methodology, Geneva.

## Submit your next manuscript and get advantages of OMICS Group submissions

### Unique features:

- User friendly/feasible website-translation of your paper to 50 world's leading languages
- Audio Version of published paper
- Digital articles to share and explore

### Special features:

- 200 Open Access Journals
- 15,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, DOAJ, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: [www.omicsonline.org/submission](http://www.omicsonline.org/submission)