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SHORT COMMUNICATION

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## Simultaneous HPLC method for determination of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline in veterinary drugs

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### Abstract

Lakkanatinaporn, P. and Matayatsuk, C.

**Simultaneous HPLC method for determination of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline in veterinary drugs**

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A simple HPLC method has been developed for the separation and determination of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline in veterinary preparations. Both drugs were separated well on a Kromasil C<sub>18</sub> column (5  $\mu$ m, 150  $\times$  4.6 mm) using a mixture of acetonitrile and 0.5% triethylamine in 1% acetic acid, pH 3 (18:82, v/v) as the mobile phase at the flow rate of 1.5 ml/min. The presence of both substances was monitored by UV absorption detection at 271 nm. The retention times of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline were 3.2 and 16.0 min, respectively. The performance of the developed method was tested. Linear responses of both drugs were achieved between 48-145% of labeled amount over the concentration ranges of 35-101  $\mu$ g/ml and 102-306  $\mu$ g/ml for sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline with correlation coefficients ( $R^2$ ) of 0.9980 and 0.9998, respectively. Accuracy expressed in term of recoveries were 101.4  $\pm$  1.21% (n=6) for sodium trimethoprim phenylpropanol disulphonate and 99.7  $\pm$  0.92% (n=6) for sodium sulfaquinoxaline. Precision of the method in terms of the relative standard deviation is not more than 2% in all cases. These figures of merit indicated the validity of the developed method.

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**Key words :** trimethoprim, sulfaquinoxaline, HPLC, veterinary preparations

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## บทคัดย่อ

พวงแก้ว ลักษณพินพ์ และ ชุติมา มัชัยสกสุข

การวิเคราะห์ปริมาณ sodium trimethoprim phenylpropanol disulphonate และ sodium sulfaquinoxaline ในยาสัตว์โดยวิธี HPLC

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วิธีโครมาโทกราฟีของเหลวสมรรถนะสูงอย่างง่ายได้ถูกพัฒนาขึ้น เพื่อทำการแยกและวิเคราะห์ปริมาณโซเดียม ไตรเมตโซพริม ฟินิลโพรพานอล ไดชัลไฟเนต และโซเดียม ชัลฟัคิวโนกซอลีน ในตัวรับยาสัตว์ ยาทั้งสองชนิด แยกออกจากกันได้ด้วยคอลัมน์โกรามาชิล ซี 18 โดยมีอะซิโตไนโตร์ และ 0.5% ไตรเอทิลเออเมิร์นใน 1% อะซิติก แอซิด พีเอช 3 อัตราส่วน 18:82 เป็นเฟสเคลื่อนที่ ยาทั้งสองถูกจัดด้วยเครื่องวัดการดูดกลืนและยูวีที่ความยาวคลื่น 271 นาโนเมตร เวลาที่ใช้เดี่ยม ไตรเมตโซพริม ฟินิลโพรพานอล ไดชัลไฟเนต และโซเดียม ชัลฟัคิวโนกซอลีน ถูกชี้แจงออกจากเฟสอยู่กับที่คือ 3.2 และ 16.0 นาที ตามลำดับ ได้ทำการตรวจสอบความถูกต้องของวิธีที่พัฒนา พบการตอบสนองที่เป็นเส้นตรงในช่วง 48-145% ของปริมาณที่ระบุบนผลลัภในช่วงความเข้มข้น 35-101 ไมโครกรัม/มล และ 102-306 ไมโครกรัม/มล สำหรับโซเดียม ไตรเมตโซพริม ฟินิลโพรพานอล ไดชัลไฟเนต และโซเดียม ชัลฟัคิวโนกซอลีน โดยมีสัมประสิทธิ์เชิงเส้น ( $R^2$ ) เท่ากับ 0.9980 และ 0.9998 ตามลำดับ ความถูกต้องของวิธี (accuracy) และในรูปของค่าการกลับคืน (recovery) เท่ากับ  $101.4 \pm 1.21\%$  ( $n=6$ ) สำหรับโซเดียม ไตรเมตโซพริม ฟินิลโพรพานอล ไดชัลไฟเนต และ  $99.7 \pm 0.92\%$  ( $n=6$ ) สำหรับโซเดียม ชัลฟัคิวโนกซอลีน ความแม่นยำ (precision) ของวิธีวิเคราะห์มีค่าเบี่ยงเบนมาตรฐานไม่เกิน 2% ค่าเหล่านี้แสดงถึงความน่าเชื่อถือของวิธีวิเคราะห์ที่พัฒนาขึ้นมา

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Sulfaquinoxaline is a member of the sulfonamides, bacteriostatic anti-microbials that interfere with the biosynthesis of folic acid in bacterial cells. The inhibitory mechanism of sulfonamides group is the competition with para-aminobenzoic acid (PABA) for incorporation into dihydrofolic acid. By replacing the PABA molecules in dihydrofolic acid, they prevent the formation of folic acid required for nucleic acid synthesis and multiplication of the bacterial cell (Prescott *et al.*, 1993; Applegate, 1983).

Diaminopyrimidines, for example ormethoprim and trimethoprim, are also bacteriostatic antimicrobials that block a step in folate production just subsequent to that affected by the sulfonamides. Potentiated sulfonamide, the combination of a sulfonamide and diaminopyrimidine, can produce a synergistic effect on bacteria. The combined and synergistic activities of the two agents in each type of potentiated sulfonamide produce antibacterial activity against a wide range

of infections caused by gram-positive and gram-negative bacteria, some protozoa, and some anaerobes under certain conditions (Indiveri *et al.*, 1986; Nicolle, 2003; Poros-Gluchowska *et al.*, 2003; Conner, 1997; Dummer, 1990). The combination of sodium trimetroprim phenylpropanol disulphonate and sodium sulfaquinoxaline is an example of potentiated sulfonamide preparation for animal use.

The suitable analytical method is important for quality control of potentiated sulfonamide products. Liquid chromatograph (LC) has been applied to the determination of sulfaquinoxaline (USP 26) and trimethoprim (USP 26). Since the combination preparation of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline is not available in the United States Pharmacopeia (USP) and other pharmacopeias, the LC assays recommended by USP for sulfaquinoxaline and trimethoprim have to use different mobile phase conditions. In USP 26, HPLC con-

dition used for determination of trimethoprim or trimethoprim in the combination with sulfamethoxazole is reversed phase column ( $C_{18}$ ) with the mobile phase containing a mixture of water, acetonitrile and triethylamine, pH  $5.9 \pm 0.1$  (1400:400:2, v/v). A reversed phase  $C_{18}$  column with mobile phase containing water, acetonitrile, glacial acetic acid, tetrahydrofuran and ammonium hydroxide (583:400:10:5:2) was used to determine sulfaquinoxaline in oral suspension (USP 26). This research aimed to develop a simple isocratic LC method for determination of both drugs simultaneously. The chemical structures of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline are displayed in Figure 1.

## Experimental

## Chemicals

Acetonitrile, HPLC grade was purchased from Labscan Co Ltd., Thailand. Triethylamine

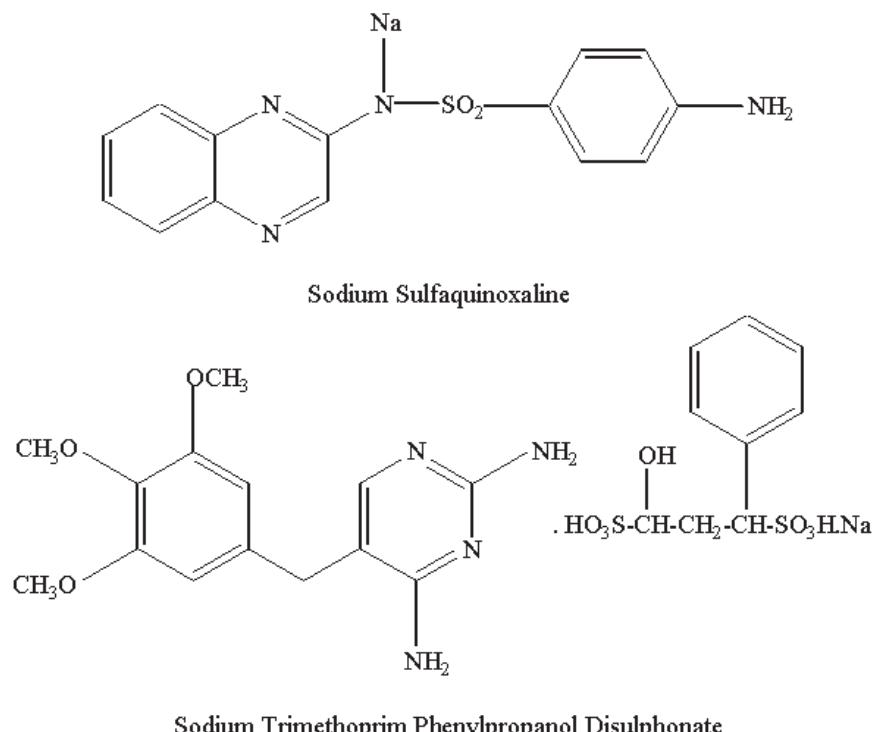
and sodium sulfaquinoxaline were from Fluka, USA. Sodium trimethoprim phenylpropanol disulphonate was obtained from Industrial GMB s.a. and glacial acetic acid was purchased from J.T. Baker, USA.

## Instrumentation and conditions

The HPLC set was Shimadzu LC-10A (Japan) equipped with LC-10AD pump, a SPD-10A UV-Vis variable wavelength detector (set at 271 nm), LC work station class LC-10 and Rheodyne 7125 injector with 20  $\mu$ l loop (USA). The mobile phase was a mixture of acetonitrile and 0.5% triethylamine in 1% acetic acid pH 3 (18:82 v/v). The flow rate was 1.5 ml/min. The reversed phase C<sub>18</sub> column (Kromasil C<sub>18</sub>, 5  $\mu$ m, 150 $\times$ 4.6 mm.) was purchased from Hypersil, England.

## Standard solution

Twenty milligrams of sodium trimethoprim phenylpropanol disulphonate and sixty-five milli-



**Figure 1. Chemical structures of sodium sulfaquinoxaline and sodium trimethoprim phenylpropanol disulphonate**

grams of sodium sulfaquinoxaline were accurately weighed and transferred to a 50 ml volumetric flask. Then, 30 ml of water was added to dissolve the drugs, the solution was diluted to volume with water and mixed. Five milliliters of the solution was pipetted into a 25 ml volumetric flask, the solution was diluted to volume with water and mixed. Twenty microliters of this solution was injected into the chromatograph for analysis.

### Sample solution

About 100 mg portion of sample powder was accurately weighed and transferred to a 50 ml volumetric flask. Thirty milliliters of water was added to dissolve the powder, then diluted to volume with water and mixed. Five milliliters of the solution was pipetted into a 25 ml volumetric flask, the solution was diluted to volume with water and mixed. Twenty microliters of this solution was injected into the chromatograph for analysis. The quantities of the two drugs were calculated by comparing with the peak areas of authentic compounds.

### Validation of the method

#### Linearity

The series concentration of the two drugs, 35, 50, 67, 84 and 101  $\mu\text{g}/\text{ml}$  for sodium trimethoprim phenylpropanol disulphonate and 102, 153, 204, 255 and 306  $\mu\text{g}/\text{ml}$  for sodium sulfaquinoxaline, were prepared. Twenty microliters of each solution was injected into the chromatograph. The peak areas of each drug were plotted versus various concentrations.

#### Precision

Three standard mixtures of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline in water were prepared in the concentrations of 67.52  $\mu\text{g}/\text{ml}$  and 196.4  $\mu\text{g}/\text{ml}$  (96% of labeled amount), 84.40  $\mu\text{g}/\text{ml}$  and 240.8  $\mu\text{g}/\text{ml}$  (121% of labeled amount), and 101.28  $\mu\text{g}/\text{ml}$  and 280.1  $\mu\text{g}/\text{ml}$  (145% of labeled amount), respectively. Three replicate injections of each mixture were introduced to the HPLC system. The relative standard deviations were calculated.

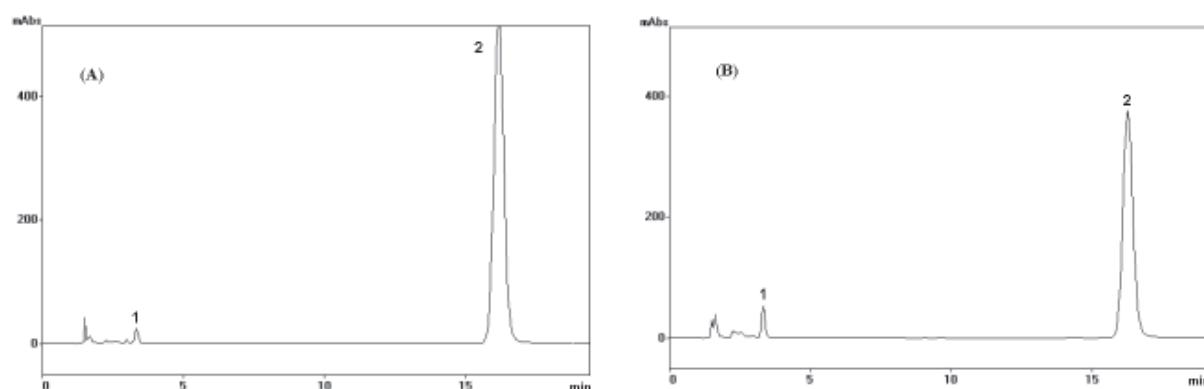
#### Accuracy

The accuracy of the method was determined by using standard addition method of six independent determinations. About 80  $\mu\text{g}/\text{ml}$  of sodium trimethoprim phenylpropanol disulphonate and about 260  $\mu\text{g}/\text{ml}$  of sodium sulfaquinoxaline were added into the analyzed sample solution. The accuracy is expressed in terms of the recovery of the added standard found.

### Results and Discussion

To date, there are no reported HPLC methods for the simultaneous determination of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline. Therefore, a simple, accurate and precise HPLC method for simultaneous determination of both drugs was developed. Preliminary isocratic studies on a Kromasil C<sub>18</sub> column with different mobile phase combinations of acetonitrile (18%, 20% and 25%) in 1% acetic acid containing triethylamine (0.1%, 0.2% and 0.5%) were considered. The higher the concentration of acetonitrile the shorter the retention times of both drugs but the peak of sodium trimethoprim phenylpropanol disulphonate overlapped with the front peak. With increased concentration of triethylamine, sharp peaks of both drugs were obtained. The optimum composition of the mobile phase was found to be acetonitrile: 0.5% triethylamine in 1% acetic acid pH 3 (18:82 v/v). Good separation was obtained and is illustrated in Figure 2. The retention times of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline were 3.2 and 16.0 minutes, respectively. The precision of retention times for sodium trimethoprim disulphonate and for sodium sulfaquinoxaline were 0.69% and 0.57% respectively. This is a good precision by USP standards (not more than 2%). However, the retention times vary with the change of temperature (23-27°C); the lower the temperature the longer the retention time especially for sodium sulfaquinoxaline (15.0-16.3 min).

To confirm the suitability and effectiveness of the operating system, the repeatability (%RSD),



**Figure 2.** The typical chromatograms of (A) standard mixture of trimethoprim phenylpropanol disulphonate sodium (1) and sulfaquinoxaline (2) and (B) drug sample.

tailing factor (T), number of theoretical plates (N), were evaluated for system suitability test. The resolution between sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline was not less than 5. These parameters were calculated from nine replicate determinations (3 concentrations, 3 replicates each) of working standard solutions. The results obtained are reported in Table 1 and are in concurrence with the USP requirement. The relative standard deviation (repeatability) is not more than 2%. Tailing factor is not more than 2. Number of theoretical plates is not less than 2000.

The linearity relationship between the peak areas and the contents of sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline were obtained throughout the concentration ranges studied. Good straight line correlations between the concentration and the

peak area were obtained with correlation coefficients of 0.9980 and 0.9998 for sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline respectively.

The accuracy of the method was determined by using standard addition method of six determinations, three replicate injections of each at the level of 100% of the test concentration. The accuracy was expressed in terms of the recovery. The precision of the method was determined in terms of the relative standard deviation of the recoveries. They were not more than 2% for all cases. The results are shown in Table 2.

### Conclusion

The developed LC method was successful for simultaneous determination of sodium trimethoprim phenylpropanol disulphonate and

**Table 1.** System suitability test

Compound	% RSD		T	N
	Retention time	Area		
TMP	0.69	1.13	1.06	3083
SFQ	0.57	0.78	1.06	9655

T = tailing factor (n=9)

N = number of theoretical plates (n=9)

%RSD = relative standard deviation (n=9)

**Table 2. Accuracy and precision studies.**

No	TMP*			SFQ*		
	Amount Added ( $\mu\text{g}/\text{ml}$ )	Amount found ( $\mu\text{g}/\text{ml}$ )	%Recovery	Amount Added ( $\mu\text{g}/\text{ml}$ )	Amount found ( $\mu\text{g}/\text{ml}$ )	%Recovery
1	80.00	82.25	102.81	258.00	255.99	99.22
2	79.20	79.89	100.87	258.00	255.29	98.95
3	80.40	79.91	99.39	260.80	257.88	98.88
4	80.00	80.91	101.13	258.80	261.67	101.11
5	79.60	81.00	101.76	258.80	260.28	100.57
6	79.60	81.45	102.33	256.80	255.67	99.56
Average			101.38			99.72
SD			1.21			0.92
%RSD			1.20			0.92

\*TMP and SFQ are trimethoprim phenylpropanol disulphonate sodium and sulfaquinoxaline sodium, respectively.

sodium sulfaquinoxaline. There are no reported HPLC methods for simultaneous determination of both drugs. The combination preparation of both drugs is not available in USP and the analysis of the two drugs described in the United State Pharmacopeia uses different HPLC conditions. Therefore, the LC method with the simple isocratic elution system was proposed to simultaneously determine sodium trimethoprim phenylpropanol disulphonate and sodium sulfaquinoxaline instead of separate assays of the individual components. Thus, the proposed method is convenient for routine use and less time consuming. In addition, tests showed good performance and system suitability of the developed method.

#### References

Applegate, J. 1983. Clinical pharmacology of sulfonamides. *Mod Vet Pract*, 667-669.

Conner, B.A. 1997. Cyclospora infection: a review. *Ann Acad Med Singapore*, 26(5); 632-636.

Dummer, J.S. 1990. *Pneumocystis carinii* infections in transplant recipients. *Semin Respir Infect.*, 5(1); 50-57.

Indiveri, M.C. and Hirsh, D.C. 1986. Susceptibility of obligate anaerobes to trimethoprim-sulfamethoxazole. *J Am Vet Med Assoc*, 188(1): 46-48.

Nicolle, L.E. 2003. Urinary tract infection: traditional pharmacology. *Dis Mon.*, 49(2); 111-128.

Poros-Gluchowska, J. and Markiewicz, Z. 2003. Antimicrobial resistance of *Listeria monocytogenes*. *Acta Microbiol Pol.*, 52(2); 113-129.

Prescott, J.F. and Baggott, J.D. 1993. Antimicrobial therapy in veterinary medicine, 2<sup>nd</sup> ed. Ames, Iowa: Iowa State University Press, 119-126.

The United States Pharmacopeia - The National Formulary (USP26-NF21), Asian Edition 2003 United States Pharmacopeia Inc, Rochelle, MD, USA., 1738-1742.