Egypt. J. Anal. Chem., Vol. 17, pp. 114-126.(2008)

HPLC Methods for Stability-Indication of Sulfaguanidine and Pharmacokinetic Study of Sulfaquinoxaline Sodium.

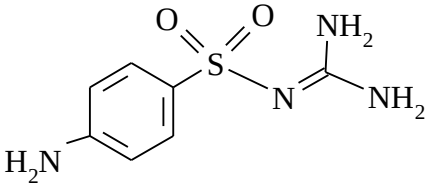
O. Abdel-Sattar, N. EI-Abasawy, S. Abdel-Razeq*, K.Abdel-Salam, N. Rashed and M. Ismail.

Analytical Chemistry Department, Faculty of Pharmacy (Boys and Girls), Al-Azhar University, Cairo, Egypt. Corresponding Author; e-mail razeqhegab@ yahoo.com

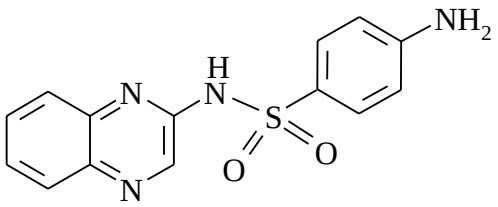
Summary: Two simple and accurate RP-HPLC methods were developed, one as stability-indicating for the determination of sulfaguanidine in presence of its degradate and the other for pharmacokinetic study of sulfaquinoxaline sodium in rabbit plasma.In the first, sulfaguanidine was degraded into p-aminobenzene sulfonic acid by reflux with 1 N HC1 for 6 h. Then the selective determination of intact drug in presence of its degradate was carried out by separation onto Econosphere C_{18} column at ambient temperature using methanol-H_2O (1:1 v/v) as mobile phase at flow rate of 0.5 mL min^{-1} and UV detection at 265 nm. The retention time was 6.35±0.05 and 4.69±0.04 min for the intact drug and its degradate, respectively. Calibration curve was obtainted in the range of 5-35 μg mL^{-1} and the method proved its specificity by determining the intact drug in presence of up to 80% of its degradate with a mean recovery of 99.6±1.38%. The second method involved extraction of sulfaquinoxaline sodium from rabbit plasma using methanol and separation onto the above column using a mobile phase of CH_3OH-0.05 M NaH_2PO_4-H_3PO_4 (10:90:2 v/v/v) at a flow rate of 2 mL min^{-1} and UV detection at 252 nm to obtain a resolved peak at 3.65±0.08 and 1.42±0.02 min, for drug and endogenous plasma components, respectively. The linearity range was 5-40 μg mL^{-1}. The pharmacokinetic study of sulfaquinoxaline sodium after individual oral dose (100 mg/kg) for rabbits showed a C_{max} of 14.25±1.04 μg mL^{-1}, T_{max} of 3 h, $t_{1/2}$ of 4.44±1.10 h and AUC (0-24) of 60.3± 1.32μg h mL^{-1}.

Introduction

Sulfaguanidine (SG); p-aminobenzene-sulfonylguanidine and sulfaquinoxaline sodium (SX-Na); 4-amino-N-2-quinoxalinyl benzene sulfonamide are antibacterial sulfonamides[1,2]. Both are used in veterinary medicine where SG is used for intestinal infections, and SX-Na in the treatment of outbreaks of coccidiosis, cholera and typhoid[3].



Sulfaguanidine



Sulfaquinoxaline

SX-Na is officially determined in the USP 2004 by HPLC[4]. Several methods have been reported for the analysis of both drugs including spectrophotometric[5-13],electrochemical[14,15],titrimetric[16,17]and TLC[18,19], in addition to numerous HPLC methods for their determination in pharmaceuticals, eggs, milk and animal tissues[20-29].

Surveying the literature in hand no mention was found about stability study for SG. Thus the aim of the present work is to develop a simple and sensitive RP–HPLC procedure to check the stability of SG and to monitor blood levels following administration of therapeutic doses of SX-Na.

Experimental

Samples

Reference standards sulfaguanidine SG (99.3%) and sulfaquinoxaline sodium SX–Na (99.3%) were kindly supplied by Egyptian company for chemicals and pharmaceuticals (ADWIA), 10th of Ramdan city, Egypt .Sulfaguanidine WSP powder labelled to contain 100 gm SG g and sulfaquinoxaline sodium 25% WSP, the products of ADWIA were purchased from the local market .

Degraded Sample- 50 mg of pure SG powder was refluxed with 50 mL 1 N HCl acid for 6 h. After cooling, the solution was adjusted with 2 N NaOH to pH 3 and evaporated to dryness under vacuum. Residue was extracted three times, each with 25 mL methanol, then filtered into a 100 mL volumetric flask and adjusted to volume with methanol. The obtained solution assumed to contain the degradate product derived from (0.5 mg mL^{-1}) SG was analyzed by the proposed HPLC procedure.

Standard solutions- Stock solution of pure SG, 0.5 mg mL $^{-1}$ was prepared in methanol and found to be stable for 7 days either at room temp. or in refrigerator. Stock solution of pure SX–Na, 0.1 mg mL $^{-1}$ in methanol was prepared and found to be stable for 24 h at room temperature and for 7 days in refrigerator.

Chemicals and reagents

All reagents used were of analytical grade and solvents were of HPLC grade Water used throughout the procedure was freshly double distilled .

Instruments

LDC Analytical HPLC Chromatograph (Milton Roy, USA) was used. It consists of constaMetric 4100 solvent delivary system, spectroMonitor 3200 UV detector and D 2500 computing integrator. 25-μL Hamilton syringe (for injection), Vector IR spectrophotometer (8201 PC) and Varian Chemini, NMR spectrophotometer (90 MHz) were used.

Procedure for SG

a.Linearity- Standard solutions were prepared by diluting the stock drug solution (0.5 mg mL^{-1}) with methanol so as to contain 0.05–0.35 mg of SG. Then 20 μL of each solution was injected on Econosphere C18 column using a mobile phase of methanol-water (1:1 v/v), flow rate of 0.5 mL min^{-1} and UV detection at 265 nm. The peak area values were plotted as a function of drug concentrations in μg mL^{-1} to obtain the calibration curve.

b. Mixture of intact and degraded SG- Aliquot volumes of pure SG (0.5 mg mL^{-1}) containing 0.315-0.07 mg of the intact drug were transferred into a series of 10-mL volumetric flasks containing 0.035-0.28 mg of degraded SG and diluted to the volume with methanol. 20 μL of each obtained solution was injected into the HPLC column and the above mentioned conditions were followed. The intact drug concentrations were calculated from the corresponding regression parameters.

c.Application to Pharmaceutical Preparation- Weighed amount of sulfaguanidine W.S.P. powder equivalent to 50 mg sulfaguanidine was transferred into a 100-mL volumetric flask, dissolved and diluted to the volume with methanol. The obtained solution labeled to contain (0.5 mg mL^{-1}) of SG was analysed as detailed under "Linearity". The concentrations of the drug were calculated from the appropriate regression parameters.

Procedure for SX-Na

a. Blank Plasma Preparation- Blank plasma was prepared from heparinized whole-blood samples collected from healthy rabbits and stored frozen. After thawing, 0.5 mL of rabbit plasma was transferred into a glass stoppered 15 mL centrifuge tube containing 1 mL of methanol. After mixing for 30 sec, the mixture was centrifuged for 10 min at 5000 rpm and then 20 μL of the protein-free supernatant was injected into the chromatographic system.

b. Linearity- Aliquots of standard drug solution (0.1 mg mL^{-1}) containing 0.05-0.4 mg of SX-Na were introduced into a series of 10-mL volumetric flasks and adjusted to volume with methanol. 1mL of each concentration was transferred into 15 mL glass stoppered centrifuge tube containing 0.5 mL rabbit plasma. Each tube was mixed for 30 sec and centrifuged for 10 min at 5000 rpm. 20 μL injection was made for each clear supernatant and chromatographed on the above column using a mobile phase of methanol-0.05M NaH_2PO_4-H_3PO_4 (10:90:2 v/v/v), flow rate of 2mL min^{-1} and eluent was monitored at 252 nm. Calibration curve was constructed by plotting the peak area against drug concentration in μg mL^{-1}.

c.Clinical Study- Four male rabbits were individually orally administered a dose of sulfaquinoxaline sodium 25% W.S.P. (100 mg/Kg) after overnight fasting. Blood samples were collected from the contralateral vein before dosing and after dosing at different time intervals (0.5-24 h), transferred to pre-chilled heparinized tubes and immediately centrifuged at 5000 rpm for 10 min. and stored frozen until analysed by HPLC following the procedure mentioned

under "linearity". These frozen samples were found to be stable for 7 days when kept frozen as compared with freshly prepared samples.

Results and Discussion

Although SG and SX-Na are stable in neutral medium and alkaline medium, yet they are hydrolysed in acid medium.[4] The degradation and detection of the degradate together with the selective determination of intact drug by the proposed HPLC was only successful for SG. Thus, SG was heated with 0.1-1.5 N HCl for 1-7 h. Complete degradation of the drug was obtained uponrefluxing with 1 N HCl for 6h.Whereby it was hydrolysed into p-amino-benzenesulfonic acid as represented in Fig. (1).

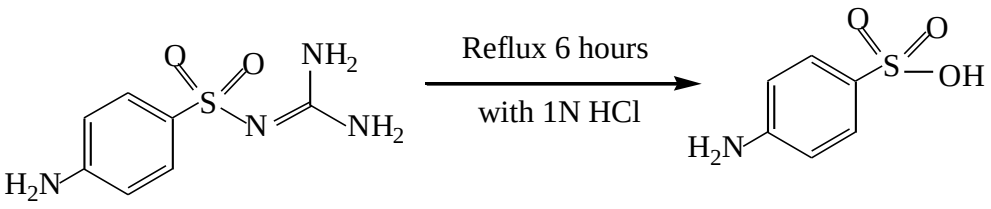


Fig. 1. Degradation of Sulfaguanidine

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To confirm this degradation the acid solution after complete hydrolysis was cooled, brought to pH 3 with 2 N NaOH, evaporated under vacuum and extracted with methanol. The methanolic solution was tested by TLC on silica gel 60 GF_{254} plate, developed with methanol-chloroform (1:1 v/v) and detection was monitored at 254 nm. Only one spot was observed for the degradate at R_f of 0.52, while that of intact drug at R_f of 0.72; confirming complete degradation of the drug. IR spectra showed two forked bands at 3489.8 to 3221.1 cm^{-1} or 3466.2 to 3185.9 cm^{-1} (-NH stretching); and two sharp bands

at 1307.7 and 1130.8 cm^{-1} or 1301.4 and 1123.7 cm^{-1} (-SO_2 stretching) for intact drug or its degradate, respectively. Also ^{1}HNMR spectrum of pure SG showed singlet of four protons overlapped on the doublet of the aromatic protons at 6.54 to 6.52 ppm due to guanidine protons which disappeared on deuteration. However that of its acid degradate showed singlet at 7.21 due to –SO_3H disappeared by D2O; giving evidence for hydrolysis of SO_2–N= bond.

HPLC for SG

Different chromatographic conditions affecting the chromatographic separation of SG from its degradate were optimized. Several mobile phases were tried including acetonitrile-H_2O, acetonitrile-H_2O-phosphoric acid and acetonitrile-methanol in different ratios. Best separation was carried out on Econosphere C_{18} column using a mobile phase consists of methanol-H_2O (50:50 v/v) at flow rate 0.5 mL min^{-1} and UV detection at 265 nm. Whereby the intact drug and its degradate were eluted at 6.35 $\pm$ 0.05 min and 4.69 $\pm$ 0.04 min, respectively, Fig.2.

HPLC for SX–Na

The chromatogram obtained from blank and plasma samples containing SX–Na is presented in Fig. 3. which declares retention times of 3.65±0.08 and 1.45±0.02 min for the drug and endogenous plasma components, respectively. Such separation was affected on Econosphere C_{18} column using a mobile phase of methanol–0.05 M NaH_2PO_4– H_3PO_4 (10:90:2 v/v/v) at a flow rate of 2 mLmin^{-1} and UV detection at 252 nm .

Method of Validation

The linearity was checked by relating area under the peak to drug concentration. Beer's law was found to be obeyed over the range 5-35 or 5-40 μg mL^{-1} of SG or SX-Na, respectively and regression parameters are given in Table (1). The LOD and LOQ were found to be 1.22 and 4.06 μg mL^{-1} or 1.44 and 4.8 μg mL^{-1} for the two mentioned drugs, respectively. The accuracy and precision for SG were assessed using 9 determinations over 3 concentrations

(10-30 μg mL^{-1}) where RSD ranged between 0.16 to 0.25% or 0.2 to 0.3% for intraday and interday analysis, Table (1). In addition, different plasma samples spiked with SX-Na (10-40 μg mL^{-1}) were analysed by HPLC, the intra- and interday RSD were found to be 0.10-0.72 and 0.20-1.23% respectively, Table (1).

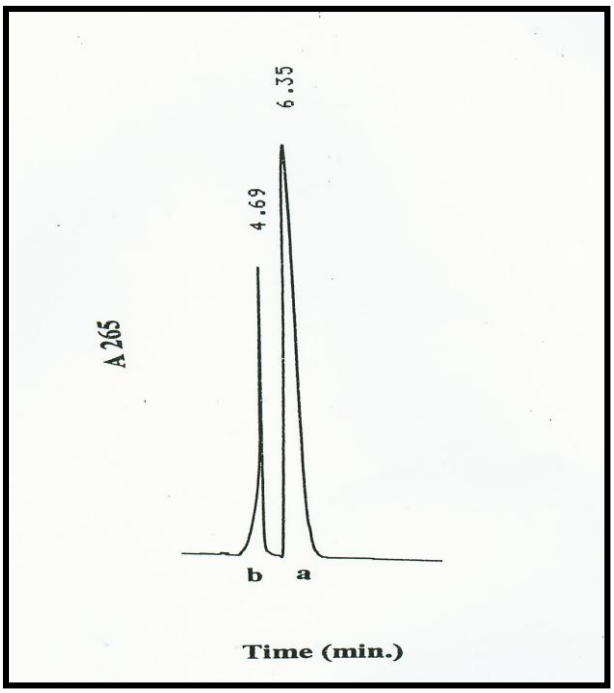

Fig. 2. HPLC chromatrogram of mixture of (a) intact sulfaguanidine, 25 μgm^{-1} and (b) degradate derived from 25 μg ml^{-1} sulfaguanidine.

The specificity of HPLC procedure for SG was assured by applying it to laboratory–prepared mixtures of intact drug and its degradate.Table (2) revealed that, intact SG can be determined selectively in presence of up to 80% of its degradate with mean % recovery of 99.5±1.47 % .

The Application to Pharmaceutical Preparation of SG

proposed HPLC procedure was applied for determination of SG in sulfaguanidine WSP, where a mean recovery of 99.0+1.31%; was obtained. Statistical analysis of these results compared with those obtained by a reported method[2] indicated no significant difference between the two methods with respect to accuracy and precision at 95% confidence limits[31], Table (2).

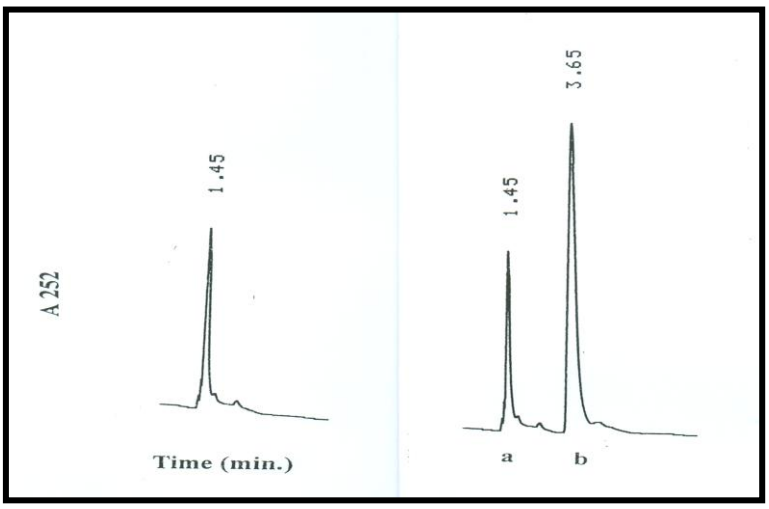

Fig. 3. HPLC chromatograms of (a) rabbit blank plasma and (b) rabbit plasma spicked with sulfaquinoxaline sodium at 252 nm

Table (1): HPLC Data for the Determination of Sulfaguanidine and sulfaquinoxaline by the Proposed HPLC Procedure.

	SG	SX-N
Linearity Range (μg ml^{-1})	5 - 35	5-40
Working Range (μg ml^{-1})	5 – 35	5-40
λ_{max} nm	265	252
LOD (μg ml^{-1})	1.22	1-44
LOQ (μg ml^{-1})	4.06	4-8
Response Factor ± SD	0.097 ± 4.706 E-03	0.017±5.54 E-04
Regression Parameters:		
- Slope ± SD (S_b)	0.0949 ± 3.228 E-03	0.018±4 E-04
- Intercept ± SD (S_a)	0.0285 ± 6.752 E-02	-0.02 ±2.46 E0.02
- SD Residual (S_{xy})	1.406 E– 01	2.899E-02
Correlation Coefficient (r^2)	0.9965	0.9974
Accuracy *		
Intraday	98.1-101.1	97.5-98.9
Interday	97.7-101.6	99.2-101.4
Precision*		
Intraday	0.16-0.25	0.1-0.72
Interday	0.20-0.30	0.83-1.23

* 9 determinations

The absolute recovery of SX-Na

The mean peak area of triplicate analysis of SX-Na from spiked rabbit plasma were compared with standard solutions of the drug in methanol at 5-40 μg mL^{-1} concentrations . Table (3) indicated that the absolute recovery was 86.7±0.67%.

Table (2): Determenation of Sulfaguanidine in Mixture with its Degradation Product and in its Pharmaceutical Preparation

Mixture Intact and Degraded Sulfaguanidine			Parameter	Sulfaguanidine W.S.P	
Intact ($\mu g\ ml^{-1}$)	Degradate ($\mu g\ ml^{-1}$)	Intact Recovery %		Proposed HPLC Procedure	Repored Procedure[2]
			Concentration Range($\mu g\ ml^{-1}$)	5-35	2-12
31.5	3.5	100.1			
28.0	7.0	99.8	N	5	5
24.5	10.5	98.9	Mean Recovery%	99.0	99.6
21.0	14.0	97.4			
17.5	17.5	98.2	SD	1.31	0.82
14.0	21.0	100.8	Variance	1.716	0.672
10.5	24.5	98.9	t-Student Test	0.869 (2.306)	
7.0	28.0	102.0			
Mean ± SD%		99.5 ±1.47	F	2.55(6.39)	-

- Figures in Parenthesis are the theoretical t and F values at P=0.05.
- Ref.(2): SG Was determined by UV measurement at 259 nm of its aqueous alkali solution.

Pharmacokinetic Study of SX-Na

The pharmacokinetic parameters of SX-Na were determined after administration of an oral dose of SX-Na 25% WSP powder (100 mg/kg) to four male rabbits after overnight fasting. Fig. 4. showed the change of mean plasma concentration where it increased to reach a maximum of about 15 $\mu g\ mL^{-1}$ at 3h after administration and then slowly decreased to a level $\leq$ 2.5 $\mu g\ mL^{-1}$ by 24 h after administration. C_{max} was 14.25$\pm$1.04 $\mu g\ mL^{-1}$, T_{max} was 3h and half-life ($t_{1/2}$) was 4.44$\pm$1.10 h. The area under the plasma concentration-time curve from time zero to 24 h ($AUC_{(0-24)}$) was calculated to be 60.3$\pm$1.32 $\mu g\ h\ mL^{-1}$ using the linear trapezoidal rule[32,33].

Table (3): Absolute Recovery of Sulfaquinoxaline Sodium From Plasma.

Taken conc.(μg ml^{-1})	Found conc. (μg ml^{-1})		Recovery %
	Standard	Spiked Plasma	
5	4.89	4.22	86.3
10	9.91	8.66	87.4
15	14.82	12.93	87.2
20	19.78	17.15	86.7
25	24.27	20.94	86.3
30	29.64	25.43	85.8
35	34.07	29.37	86.2
40	38.98	34.19	87.7
Mean ± SD%			86.7 ± 0.67

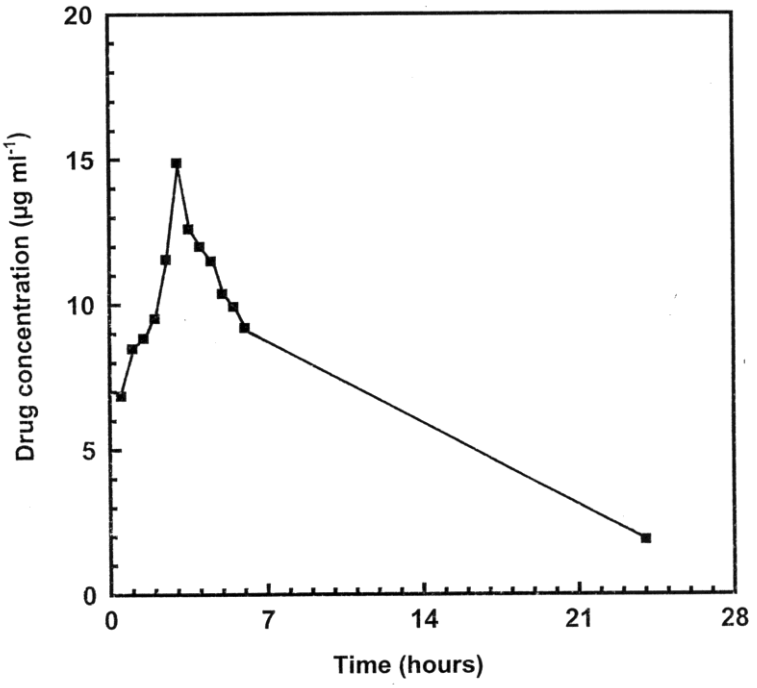

Fig. 4. Plasma concentration-time profile of sulfaquinoxaline-sodium.

Conclusion

The two HPLC procedures are simple, rapid and accurate. For sulfa-guanidine the proposed HPLC procedure is useful for the selective determination of the intact drug with the elimination of both matrix and degradation product interferences in the analysis of formulated drug. For sulfaquinoxaline sodium, the ease of extraction procedure and time saving provides a good recovery of the drug from plasma.

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