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ANTIBIOTIC PRINCIPLES FROM A *STREPTOMYCES* SPECIES AND THEIR SUB-ACUTE TOXICITY STUDIES ON HEPATIC, RENAL AND HAEMOPOIETIC SYSTEM OF RATS

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Two promising antibiotics, JF-A and JF-B were isolated from the chloroform extract of a Banglaeshi *Streptomyces* strain. The mean zones of inhibition produced by the chloroform extract (400 µg/disc), JF-A (200 µg/disc) and JF-B (200 µg/disc) against 19 pathogenic bacteria were found to be 9-50, 12-38 and 10-41 mm while those produced by a standard antibiotic, kanamycin were 11-40 mm at 30 µg/disc. MICs of JF-A and JF-B were determined to be 64 µg/ml against *Bacillus subtilis* and 64 and 128 µg/ml against *Shigella sonnei*, respectively. The extract and the antibiotics were also tested for cytotoxicity against *Artemia salina* nauplii and LC_{50} values of 23.26, 18.05 and 32.27 µg/ml were obtained. 90% mortality of shrimp nauplii was observed at 69.18, 50.12 and 110.91 µg/ml, respectively. In a potato disc bioassay, the chloroform extract at 25 µg/disc demonstrated 37.39% inhibition of crown gall tumour induced by G -ve *Agrobacterium tumefaciens* B6 and the result was statistically significant ($P < 0.05$). The sub-acute toxicity studies on the JF-A and JF-B reflected innocuous nature of these antibiotics on hepatic, renal and haemopoietic system of rats at 1 mg/kg b.w. on daily administration for 21 consecutive days. This is also confirmed by detailed histopathological studies. No mortality was observed in experimental animals.

Keywords: Streptomyces *sp., antibacterial, antitumour, cytotoxicity, sub-acute toxicity.*

INTRODUCTION

A major problem in the antimicrobial therapy is an increasing resistance in pathogenic bacteria to the classic antibacterials, such as the penicillins. A potential approach to overcome this problem is to design new and innovative agents with a totally different mode of action so that no cross-resistance with the present therapeuticals can occur. More than four thousand antibiotics were reported from microbes; the bacterial order of Actinomycetales, which includes the genus *Streptomyces*, occupies the first position regarding the number of antibiotics isolated (Reiner, 1982). *Streptomyces* is a rich source of many chemically diversified and biologically important secondary metabolites including terpenoids, steroids, alkaloids, carbohydrates, glycosides, polyketides, peptides, aromatic and straight-chain compounds (Dictionary of Natural Products, 2001). A number of these metabolites are noted for their broad range of biological activities including antibacterial, antifungal, antitumor, and immunosuppressive activities (NCI database, 2002). In search of novel antibacterial principles from microbial source we investigated a *Streptomyces* sp. collected from the soil samples of Bangladesh and recently reported a new dammarane triterpene, 16,20-diacetoxy-dammara-1,5,24-trien-3-one (Mazid *et al.,* 2002). The present work was an endeavor to screen the extractives for probable antibacterial and cytotoxic activities as well as to investigate toxicity potential with particular reference to haemato-biochemical alterations in rats.

MATERIALS AND METHODS

General experimental procedures

The ^{1}H NMR (270 MHz) spectra were recorded in $CDCl_3$ on a Bruker WH 270 spectrometer using TMS as an internal standard. The chemical shifts (δ) and coupling constants (*J*) were expressed in ppm and Hz, respectively. UV and Infrared (IR) spectra were obtained on Perkin-Elmer spectrophotometers. MS spectra were recorded at 70 eV in the EI+ mode on a Varian MAT 711 bzw mass spectrometer.

Collection of soil samples

Soil samples of the depth ranging from 0.25 to 1.5 m were collected from a wide range of areas including roadsides, construction sites, drains, agricultural wastes, food wastes, graveyards, sewage and cultivated lands of Dhaka, Faridpur, Jessore, Khulna, Sylhet and Habiganj districts of Bangladesh. These samples were tested for antagonistic species by crowded plate technique in Czapek-Dox agar medium.

Isolation and identification of organism

A grayish black organism with an orange-red secretion was

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identified as a *Streptomyces* sp. based on its morphological and biochemical characteristics after 3rd day of incubation in Czapek-Dox medium (Holt *et al.*, 1994). However, the observations did not facsimile any of the known *Streptomyces* sp. recommended in the Bergey's manual of determinative bacteriology indicating the organism to be a new species or a physiological variant. The organism was isolated as pure culture using serial dilution technique (Pelczar *et al.*, 1986).

Selection of optimum culture conditions

For isolation of antibacterial metabolites, the optimal conditions for bacterial growth were ascertained using different broth media (Czapek-Dox, starch casein broth, Jensen medium, glucose asparagine broth, yeast ext. glucose, nutrient broth), pH (3-10), temperature (30-45°C), sugar (glucose, fructose, sucrose, rhamnose, galactose, mannose, lactose and inositol) and salt concentrations (0-5%) on the basis of two parameters, *viz.*, biomass and the zone of inhibition by disc diffusion (Bauer *et al.*, 1966).

Extraction

The pure culture was grown at 37.5 °C for 10 days on a rotary shaker using the most suitable Czapek-Dox broth medium (pH 4.5). A 25-ml portion of the culture thus prepared was used to inoculate six 500 ml Fernbach flasks containing 200 ml of sterile Czapek-Dox broth acidic medium. Fermentation was carried out at 37.5 °C for 10 days after which the media turned orange-red with purple-gray, thick and uniform mat on the surface. The mycelial mat was separated and the cultured broth was centrifuged, followed by filtering the supernatant. A 100 ml filtrate was extracted thrice with chloroform (3 × 30ml). The extract was then filtered again through fresh cotton bed and concentrated to a gummy substance under reduced pressure at 40 °C using a Büchi rotary evaporator.

Table 1

Antibacterial activity of the crude chloroform extract and antibiotics JF-A and JF-B

Micro-organism	Strain No.	Diameter of zone of inhibition (mm)						
		Ext-400	Ext-200	JF-A 400	JF-A 200	JF-B 400	JF-B 200	Kan 30
Aeromonus hydrophilia	AM 10481	23	18	-	-	30	25	21
Bacillus cereus	QL 29	35	31	43	35	39	37	34
Bacillus megaterium	QL 38	35	30	42	35	36	34	32
Bacillus subtilis	QL 40	34	28	38	36	41	36	31
Escherichia coli	ATCC 25922	14	-	15	12	18	13	21
Klebsiella sp.	-	38	35	40	34	33	26	35
Pseudomonas aeruginosa	ATCC 27853	36	33	41	36	42	35	34
Salmonella paratyphi A	AM 16590	37	34	42	33	39	37	32
Salmonella paratyphi B	AM 15961	31	28	39	35	44	41	34
Salmonella typhi	AM 16406	34	31	39	32	36	32	30
Sarcina lutea	QL 166	33	27	37	31	40	37	34
Shigella boydii	ATCC 13147	35	27	38	31	40	37	33
Shigella dysenteriae	ATCC 26131	50	11	32	27	12	10	28
Shigella flexneriae	Y 976	9	-	-	-	-	-	23
Shigella sonnei	C 182	35	31	42	35	39	34	35
Staphylococcus aureus	ATCC 25923	35	29	39	33	41	38	35
Vibrio cholerae (classical)	569 B	37	29	42	38	42	37	40
Vibrio mimicus	N 1967	27	32	39	37	37	32	33
Vibrio parahemolyticus	AM 16362	13	9	9	-	9	-	11

Ext-400 and Ext-200 = Chloroform extract of a *Streptomyces* culture broth at 400 and 200 μg/disc, respectively;
JF-A 400 and JF-A 200 = Antibiotic JF-A at 400 and 200 μg/disc, respectively;
JF-B 400 and JF-B 200 = Antibiotic JF-B at 400 and 200 μg/disc, respectively;
Kan = Kanamycin (30μg/disc) and '-' indicates no sensitivity or zone of inhibition lower than 9 mm.

Table 2
Minimum inhibitory concentration (MIC) of antibiotics JF-A and JF-B against *Bacillus subtilis* and *Shigella sonnei*

Test tube No.		1	2	3	4	5	6	7	8	9	C_{MC}	C_{MI}	C_{M}
Concentration (μg/ml)		256	128	64	32	16	8	4	2	1	256	0	0
JF-A	*Bacillus subtilis*	-	-	-	+	++	++	++	++	++	-	++	-
	Shigella sonnei	-	-	-	+	++	++	++	++	++	-	++	-
JF-B	*Bacillus subtilis*	-	-	-	+	++	++	++	++	++	-	++	-
	Shigella sonnei	-	-	+	++	++	++	++	++	++	-	++	-

CMC = Medium + Compound; CMI = Medium + Inoculum; CM = Medium only
+ = Growth, - = No growth
MICs (calculated) = 64 μg/ml against *Bacillus subtilis* and 64 and 128 μg/ml against *Shigella sonnei* for JF-A and JF-B, respectively.

Table 3
Cytotoxicity of the crude chloroform extract and antibiotics JF-A and JF-B on brine shrimp nauplii

Concentration (μg/ml)	Chloroform extract	
	Mortality (%)[a]	LC_{50} (μg/ml)[b]
10	23.33 ± 0.58	23.26 (22.37-24.16)
20	33.33 ± 0.58	
40	70.00 ± 1.00	
80	96.67 ± 0.58	
160	100.00 ± 0.00	

Concentration (μg/ml)	JF-A		Concentration (μg/ml)	JF-B	
	Mortality (%)[a]	LC_{50} (μg/ml)[b]		Mortality (%)[a]	LC_{50} (μg/ml)[b]
10	26.67 ± 0.58	18.05 (17.35-18.75)	10	15.00 ± 1.00	32.27 (31.37-33.19)
20	46.67 ± 0.58		20	30.00 ± 1.00	
40	86.67 ± 0.58		40	53.33 ± 0.58	
80	100.00 ± 0.00		80	80.00 ± 1.00	
160	100.00 ± 0.00		160	100.00 ± 0.00	

[a] Values are Mean ± S.D. (n = 3); [b] Confidential limits 95% in parentheses.

Isolation of compounds

The crude chloroform extract was fractionated by column chromatography over Kieselgel 60 (mesh 70-230), and the column was eluted with EtOAc and EtOAc-MeOH mixtures of increasing polarity, with 22 fractions collected (each *ca.* 20 ml). Screening of column fractions by TLC demonstrated the presence of two prominent bands in fractions 7 to 10. These fractions were subjected to preparative TLC over Si gel PF_{254} using EtOAc-$CHCl_3$ (80:20) as developing solvent, which yielded JF-A (higher band) and JF-B (lower band). The compounds were finally eluted with EtOAc. Chromatographic analyses of JF-A and JF-B for purity revealed single chemical enteritis as quenched spot at 254 nm and greenish fluorescence at 366 nm along with trace impurities.

Studied activities

In vitro antibacterial screening (Bauer ., 1966)

Standardized disk diffusion method was employed for *in vitro* antibacterial screening against 19 strains of both Gram positive and negative bacteria collected from the Institute of Nutrition and Food Science (INFS), University of Dhaka. The experiment was conducted twice and the mean zones were recorded. The results obtained were compared with a standard antibiotic, kanamycin (30 μg/disc).

Table 4
Changes in body weight of rats following treatment with different doses of JF-A and JF-B

Groups	Dose (mg/kg b.w.)	Body Weights (gm) [a]		% Weight gain [a]	T-test (P value)
		Initial	After 21 days of drug treatment		
Control	-	176.20 ± 5.63	179.00 ± 4.64	1.61 ± 0.78	0.01
JF-A	1 (i.p.)	199.80 ± 4.55	202.20 ± 4.15	1.21 ± 0.78	0.02
JF-B	1 (i.p.)	193.40 ± 5.68	195.60 ± 4.72	1.15 ± 0.70	0.02

[a] Values are Mean ± S.D.; n =5 animals in each group
Students paired t-test for difference in initial and after 21 days of treatment.

Table 5
Effect of daily oral administration of JF-A and JF-B for 21 days on haematological changes in rats

Groups	Day	Hb (gm%)	TEC (10^6/ mm^3)	TLC (10^3/mm^3)	DLC (Count/mm^3)				Platelets (10^3/ mm^3)
					Neutrophil	Lympho-cyte	Mono-cyte	Eosinophil	
Control	1	14.54 ± 0.32	4.99 ± 0.30	11.48 ± 0.76	40.80 ± 1.92	50.40 ± 2.07	3.80 ± 0.84	3.80 ± 1.79	28.00 ± 1.90
	7	14.60 ± 0.47	5.04 ± 0.15	11.96 ± 0.74	38.80 ± 3.27	50.60 ± 1.14	4.20 ± 0.84	3.60 ± 1.14	30.10 ± 0.89
	14	14.54 ± 0.50	5.09 ± 0.15	12.08 ± 0.37	39.80 ± 2.28	51.20 ± 1.30	4.60 ± 0.55	3.80 ± 0.84	32.50 ± 1.12
	21	14.88 ± 0.35	5.03 ± 0.29	11.54 ± 0.60	40.60 ± 1.82	52.40 ± 1.67	4.60 ± 0.55	5.00 ± 0.71	35.00 ± 2.00
JF-A	1	15.22 ± 0.46	5.20 ± 0.12	11.86 ± 0.23	38.40 ± 2.07	53.00 ± 2.74	4.20 ± 0.84	3.40 ± 1.14	35.80 ± 4.87
	7	15.32 ± 0.36	5.32 ± 0.08	12.18 ± 0.28	37.00 ± 1.22	54.80 ± 1.64	4.80 ± 0.84	2.80 ± 0.84	35.80 ± 2.77
	14	15.32 ± 0.43	5.32 ± 0.16	12.46 ± 0.26**	37.20 ± 1.48	54.80 ± 2.68	4.80 ± 0.45	2.20 ± 1.10	35.40 ± 2.61
	21	15.26 ± 0.47	5.20 ± 0.24	12.68 ± 0.39*	38.40 ± 2.30	54.00 ± 3.39	4.40 ± 1.14	2.80 ± 0.84	34.80 ± 2.95
JF-B	1	14.54 ± 0.36	4.92 ± 0.28	12.08 ± 0.43	41.80 ± 1.30	51.00 ± 1.58	5.00 ± 0.71	3.40 ± 1.14	35.20 ± 2.17
	7	14.78 ± 0.33	5.15 ± 0.11	12.30 ± 0.21	40.00 ± 2.45	54.20 ± 1.92**	4.80 ± 0.84	4.20 ± 0.84	36.80 ± 2.17*
	14	14.98 ± 0.33**	5.33 ± 0.19*	12.60 ± 0.14*	40.00 ± 2.65	55.00 ± 1.00**	4.40 ± 0.55	4.40 ± 1.14	37.00 ± 2.55*
	21	14.78 ± 0.34	5.04 ± 0.18	12.40 ± 0.44	40.20 ± 2.28	53.60 ± 1.34	4.40 ± 1.14	4.20 ± 0.84	35.80 ± 3.11

Values are mean ± S.D.; n = 5 in each group.
*P < 0.05 and **P < 0.01 as compared to day 1 of experiment (before treatment).

MIC assay by serial dilution technique (Reiner, 1982)
MICs of the compounds, JF-A and JF-B were determined against a Gram-positive (*Bacillus subtilis*) and a Gram-negative (*Shigella sonnei*) bacteria. Nutrient agar and nutrient broth were used as bacteriological media.

Study of antitumor activity by potato disk bioassay (McLaughlin *et al.,* 1998)
The test materials were dissolved in DMSO. Vincristine sulphate and DMSO served as positive and negative controls, respectively. Freeze dried sample of the tumour-inducing organism, *Agrobacterium tumefaciens* B6 was collected from the laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University, Japan. The bacteria were cultured in King's B medium with all aseptic precautions (Matsuyama, 1996).

Cytotoxicity by brine shrimp lethality bioassay (Meyer *et al.,* 1982)
DMSO was used as a solvent and negative control. The assay was performed using three replicates. The concentration-mortality data were analysed by using Finney

Table 6
Effect of daily oral administration of JF-A and JF-B for 21 days on biochemical changes in male rats

Groups	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Serum bilirubin (μmol/L)	Creatinine (mg %)	Uric acid (mg %)	Urea (μmol/L)
Control	7.4 ± 0.89	11.0 ± 2.00	38.0 ± 1.87	0.66 ± 0.15	1.02 ± 0.22	6.50 ± 0.21	29.0 ± 2.45
JF-A	8.4 ± 1.52	12.8 ± 1.30	41.0 ± 1.58*	0.78 ± 0.08	1.16 ± 0.21	6.68 ± 0.13	25.2 ± 1.64*
JF-B	8.4 ± 1.52	12.2 ± 1.48	40.2 ± 1.30*	0.72 ± 0.08	1.14 ± 0.27	6.70 ± 0.26	31.6 ± 2.07

Values are mean ± S.D.
*P < 0.05, compared to corresponding control group

Probit analysis (Finney, 1971). LC_{50} values were determined with 95% confidence intervals for statistically significant comparisons of potencies. Vincristine sulphate served as the positive control.

Sub-acute toxicity studies
Sub acute toxicity studies of JF-A and JF-B were carried out in Long Evan's rats to assess their effects on morphological, gross behaviour, body weight changes as well as to find out hematological, biochemical and histopathological changes.

Fifteen healthy male rats of 6 weeks were obtained from the Animal Research Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B). The animals were acclimatized to standard laboratory conditions prior to experimentation and were divided equally into three groups. The test materials (JF-A and JF-B) were dispersed in distilled water using 1% tween®-20 as suspending agent and administered to experimental groups (1 mg/kg b.w., i.p.) for twenty one consecutive days. The control group was treated with distilled water and 1% tween®-20 only.

Cage-side observations were made which included changes in the skin and fur, eyes and mucous membrane, respiratory, central nervous system, somatomotor activity and behavioural patterns and discharge from various body orifices, appearance of any tumours on body surface, water intake and body weight changes.

Hematological studies were done on 7th, 14th and 21st day after commencement of drug administration following standard procedures (Jain, 1986). Blood was drawn from the tail veins of both control and experimental animals and, the following hematological parameters were studied: Haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count.

Biochemical profiles were studied at the end of the exposure period (21 days). Following autopsy, blood was collected, centrifuged at 400 rpm for 15 min using a WIFUNG centrifuge LABOR-50M to yield serum which was used for estimation of serum glutamate oxaloacetate transminase (SGOT), serum glutamate pyruvate transminase (SGPT) (Reitman and Frankel, 1960), serum alkaline phosphatase (SALP) serum bilirubin, creatinine (Varley, 1962), uric acid and urea.

Histopathology
All animals were subjected to full gross necropsy which included examination of the external surface of the body, the thoracic and abdominal cavities and their contents, the heart, lungs, liver, kidney and spleen were examined (macroscopic and microscopic) for any detectable abnormalities in cellular structures (degeneration and regeneration). The organs were washed with normal saline and fixed in 10% neutral buffered formalin. Slices of fixed tissues were embedded in paraffin, sectioned to a thickness of *ca.* 5 mm, mounted on glass slides, and stained with haematoxylin and eosin for histological examination (Lunal, 1968).

Statistical analysis
The statistical analyses were performed by SPSS release 9.05 for Windows™. Data were decoded, analyzed and expressed as means ± S.D. The statistical comparison between control and treated groups was done by two-tailed unpaired student's t-test (homoscedastic) whilst a paired students' test was employed when there was a natural pairing of observations in the samples, i.e. when a group was observed twice, before and after an experiment (Snedecor and Cochran, 1967). Probability (P) value of 0.05 or less ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Repetitive chromatography of the chloroform extract of a *Streptomyces* sp. broth culture afforded two interesting antibiotics, JF-A and JF-B. The structures of these were not determined because of weak ^{1}H NMR signals due to trace impurities and lack of high-resolution ^{13}C and 2D NMR data. The low-resolution ^{1}H NMR of JF-A (270 MHz, $CDCl_3$) displayed signals characteristics of a substituted aromaic moiety with aliphatic side chain. The spectrum

revealed multiplets at δ 0.81 (2H), 1.62 (1H), 2.31 (1H), 4.08 (1H), two sharp singlets at δ 0.84 (3H), 3.49 (1H), a 1H doublet centered at δ 0.98 (J = 8.1 Hz) and a 1H triplet at δ 4.30 (J = 8.1 Hz). The IR spectra showed bands corresponding to hydroxyl and/or NH (3600-3400 cm^{-1}), carbonyl (1700 cm^{-1}) and aromatic (760, 770 cm^{-1}) functionalities. The UV spectrum substantiated the presence of aromatic unsaturation (263, 340 nm). The ^1H NMR spectrum (270 MHz, $CDCl_3$) of JF-B was similar to that of JF-A. The only difference observed is the absence of a 1H singlet at δ 3.49 in the ^1H NMR spectrum of JF-B.

Different organic partitionates of the broth culture using different broth media, pH, temperature, sugar and salt concentrations were tested against a Gram positive (*B. subtilis*) and a Gram negative (*S. sonnei*) bacteria by the standardized disc diffusion method (Bauer *et al.,* 1966). The maximum antibacterial activity was observed with the chloroform solubles of the metabolites from *Streptomyces* strain grown at 37.5 °C for 10 days using Czapek-Dox broth medium (pH 4.5). The chloroform extract and the isolated antibiotics JF-A and JF-B were tested against 19 pathogenic bacterial strains comprising both gram positive and gram negative for *in vitro* antibacterial activity. The results are optimistic as tabulated in table 1. A standard antibiotic, kanamycin (30 μg/disc) was used for statistical comparison. The mean zones of inhibition produced by the chloroform extract, JF-A and JF-B at 400 and 200 μg/disc dose levels were found to be 9-50, 9-35, 9-43, 12-38, 9-44 and 10-41 mm while those produced by a standard antibiotic, kanamycin were 11-40 mm at 30 μg/disc. MICs of JF-A were found to be 64 μg/ml against *B. subtilis* and *S. sonnei* while MICs for JF-B against the same organisms were 64 and 128 μg/ml (table 2).

The chloroform extract and JF-A and JF-B demonstrated moderate cytotoxicity in a brine shrimp model. The probit regression lines for every test materials at different concentrations produced approximate linear correlation between the mortality percentage and concentration. The LC_{50} values for the crude extract, JF-A and JF-B were found to be 23.26, 18.05 and 32.27 μg/ml (table 3). In a potato disc bioassay the chloroform extract at 25 μg/disc showed 37.39% inhibition of crown gall tumours on red skinned potato tubers produced by G -ve *Agrobacterium tumefaciens* B6 and the result was statistically significant ($P < 0.05$).

In the sub acute toxicity study in rats, no mortality was recorded. The treatment groups were observed daily for any signs of toxicity and/or mortality. None of the rats exhibited tremor, convulsion or any sign of abnormal behavioural responses in treated groups. No change in fur coating, eyes and mucus membrane, respiratory as well as somatomotor activity has been observed in any of the treatment groups. There was no significant difference in the food and water consumption between the treatment groups and the control. All rats showed significant increase in body weight compared to their initial values. However there was no significant difference between the different treatment groups and the control (table 4).

Hematological changes are depicted in table 5 while biochemical changes of rats are presented in table 6. The tested materials did not vigorously alter the Hb percentage, Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC) and platelet counts in male rats at any of the dosages used in this study (table 5). However, slight changes were observed in TLC after 14 and 21 days of treatment with JF-A ($P<0.05$) and marginal alterations were also seen in Hb%, TEC, TLC, DLC and platelet counts following JF-B administration as indicated in table 5. In the blood-biochemical investigations, JF-A treated rats showed a mild but statistically significant ($P<0.05$) elevation in SALP and urea levels, while JF-B induced a marginal increment in only SALP level (table 6). No significant changes were noted in other biochemical parameters that were measured. The histopathology of heart, lungs, liver, kidney and spleen were evaluated. No significant histopathological abnormalities were seen.

Since the treated rats showed no other abnormalities and were also able to maintain body weight, it appears that the antibiotics may not produce drastic ill effects up to the dose level used (1 mg/kg b.w.).

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DETERMINATION AND QUANTIFICATION OF CETIRIZINE HCl IN DOSAGE FORMULATIONS BY RP-HPLC

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A simple, sensitive, reliable and rapid HPLC method for the determination of cetirizine hydrochloride using hyoscine butyl bromide as an internal standard has been developed. The chromatographic system consisted of Shimadzu LC-10 AT VP pump, SPD-10 AV VP with UV/visible detector and a CBM-102 Bus Module integrator. Separation was achieved on the U Bondapak 125 A C18 10μm column at room temperature. The samples were introduced through an injector valve with a 10 μl sample loop. Acetonitrile-water (1:1 v/v) was used as mobile phase, with flow rate 2 ml/minutes. pH was adjusted to 2.9 with phosphoric acid. UV detection was performed at 205 nm. The results obtained showed a good agreement with the declared content. Recovery values for cetirizine hydrochloride were 99.19 - 100.82 %. The proposed method is reliable rapid, precise, selective and may be used for the quantitative analysis of cetirizine HCl, in presence of hyoscine butyl bromide as internal standard. The method was valid was for the determination in raw materials, bulk drug and formulations. The limit of quantification was 5 - 30 nano grams, while the limit of detection was 0.4 nano grams.

Keywords: Cetirizine, hyoscine butylbromide, HPLC determination.

INTRODUCTION

Cetirizine HCl or 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-piperazin-1-yl]ethoxy]acetic acid dihydrochloride, is white or almost white powder, freely soluble in water, practically insoluble in acetone and in methylene chloride, molecular weight 461.8, molecular formula, $C_{21}H_{27}Cl_3N_2O_3$ (figure 1) [The Merck Index 2001; Martindale, The Extra Pharmacopoeia 1996; British Pharmacopoeia 2003]. Cetirizine is a piperazine derivative and metabolite of hydroxyzine, is an antihistamine, reported to be a long acting and with some mast-cell stabilizing activity. It is used for the symptomatic relief of hypersensitivity reactions including rhinitis and chronic urticaria. (Anonymous 1990; Spencer *et al.*, 1993; Barnes *et al.*, 1993; Sharpe and Shuster 1993 and Snyman *et al.*, 1994). Cetirizine is rapidly absorbed from the gastro-intestinal tract after oral administration, peak plasma concentration being attained in about one hour. It is highly bounded to plasma proteins and has an elimination half-life of about 11 hours. Cetirizine has been detected in breast milk and excreted primarily in the urine mainly as unchanged drug (Awni *et al.*, 1990 and Desager *et al.*, 1993).