Spectrophotometric, Spectrofluorimetric and High Performance Liquid Chromatographic Methods for the Determination of Hexamine in Tablets

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Spectrophotometric, spectrofluorimetric and high performance liquid chromatographic methods for the determination of hexamine in pure form and in hiprex tablet are described. Both hexamine and hiprex form stable yellow colored 3,5-diacetyl-1,4-dihydrolutidine at 100° when treated with acetylacetone in phosphate buffer (pH 6.0). The chromogen shows absorption maximum at 410 nm and a fluorescence emission maximum at 510 nm. In the case of hiprex, Beer's law linearity was observed in the concentration ranges of 10-150 μg/ml, 3-30.5 μg/ml and 0.02-0.35 μg/ml in spectrophotometric, fluorimetric and high performance liquid chromatographic determinations, respectively. The common additives usually present in the tablet did not interfere in the proposed methods.

Hexamine (1,3,5,7-tetrazatricyclo[3.3.1.1^{3,7}]decane), known variously as methenamine, formamine, aminoform, urotropin and hexamethylenetetramine, is widely used as an antiseptic agent[1]. Hiprex is the drug used in the treatment of human urinary infections due to its antimicrobial activity[2]. Various analytical methods are available in the literature for the estimation of hexamine, including UV and HPLC[3-6]. Most of the spectrometric methods reported earlier suffer from disadvantages such as higher detection limits and extraction procedures for biological fluids. In the present investigation, both hexamine and hiprex have been found to form fluorescent yellow 3,5-diacetyl-1,4-dihydrolutidine with acetyl acetone having maximum absorption at 410 nm. The outstanding feature of the methods is that this enables direct assay of the analyte and found to be free from interferences due to common excipients present in tablet.

Spectral measurements were made on a Shimadzu UV-160 double beam spectrophotometer with 1-cm silica cells. Fluorescence measurements were made on a Hitachi-Model I-650-40-fluorescence spectrophotometer. The HPLC system, Model PU 8510 equipped with MD 1515-UV-Variable wavelength detector was used for the determination under isocratic condition (acetonitrile:water, 85:15 v/v). Kya tech HIQ Sil C_{18} reversed phase column (4.6 mmx250 mm) with a mobile phase flow rate of 1 ml/min. Aqueous samples were filtered through a 0.45 μm millipore membrane. Hamilton syringe was used for making serial concentrations. Toshcon pH meter model CL-54 was used for adjusting the pH of the buffer solutions. Hiprex was obtained from 3M Healthcare Limited, England. The HPLC grade acetonitrile and water were purchased from E-Merck India Ltd, Mumbai.

A freshly prepared solution of 4% (v/v) acetyl acetone in n-propanol was used for derivatization. A standard solution of hiprex was prepared by dissolving one tablet (1.02 g) in 250 ml water. A standard solution of hexamine was prepared by dissolving 0.25 g hexamine in 100 ml water. Important parameters such as pH, temperature, time and molar ratio of reactants were optimized as suggested by Job's method. The reaction carried out at pH 6.0 and at 100° for 1 h was found to yield optimum results. Acetyl acetone in n-propanol (4 % v/v, 0.5 ml) was mixed with serial concentrations of hexamine or hiprex in sodium phosphate buffer (2.0 ml, 0.1 M, pH 6.0), and heated over an oil-bath at 100° for 60 min. The yellow derivative formed was diluted to 10 ml

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TABLE 1: OPTICAL CHARCTERISTICS AND PRECISION DATA. .

Parameters Characteristics	UV/Vis		Fluorimetry		HPLC	
	HMTA	HIPREX	HMTA	HIPREX	HMTA	HIPREX
λ_{max}(nm)/λ_{em}(nm)	410		510		410	
Stability(d)	2		2		2	
Beer's law range (μg/ml)	6-75	10-150	2-18	3-30.5	0.02-2	0.03-3.5
Limit of detection (μg/ml)	5.5	9	0.95	1.8	0.01	0.02
Limit of quantification (μg/ml)	18.3	30	3.2	6.0	0.03	0.067
Molar absorptivity ($mole^{-1}.cm^{-1}$)	3699		-		-	
Sandell's sensitivity ($\mu g/cm^2$)	2.6177		-		-	
Regression equation (Y)[a]						
Slope (B)	0.0153	0.0051	0.0233	0.0154	7.453	2.9928
Intercept (A)	-0.1156	-0.0164	0.082	-0.0005	-1.1E6	-6.2E4
Correlation coefficient (r)[b]	0.9924	0.9859	0.9952	0.9945	0.9853	0.8983
Coefficient of variation[c]	0.4531	0.6466	0.4401	0.4146	0.8286	0.8983

[a]Y=A+Bx Where x is the concentration in (μg/ml); [b]n=5; [c]Five replicates.

with phosphate buffer. UV/Vis absorption and fluorescence emission spectra were recorded after appropriate dilutions. Sharp and reproducible chromatograms were obtained in HPLC by injecting different volumes (5-25 μl) of the yellow solution obtained from serial dilutions of hexamine or hiprex. The formation of 3,5-diacetyl-1,4-dihydrolutidine, was confirmed by comparing the spectral properties of the product with those of the authentic sample. Calibration graphs were constructed by plotting the concentration of the analyte vs. peak area (HPLC), relative emission intensity at 510 nm (fluorimetry) and absorbance at 410 nm (UV/Vis).

Optical characteristics such as Beer's law limits, molar extinction coefficient, correlation coefficient, %RSD, slope and intercept of regression analysis using least square methods (Origin 5.0 professional) are summarized in Table 1. The limit of detection was calculated as proposed by Long[7]. Five replicate determinations at different concentration levels were carried out and the relative standard deviation (%RSD) was observed to be less than 1. The figures obtained point out to the good accuracy and repeatability of the methods. To examine the ruggedness of the procedure the within-day and between-day precision were evaluated by analysis of 100 μg of hexamine for four consecutive days and the percentage recoveries varied from 99.34 to 100.02.

The results obtained for the assay of tablet were compared to that of official method and presented in Table 2. The results of proposed methods were found to be comparable statistically, in terms of students 't' test and Variance ratio 'F' test, with official method. At 95% confidence level, the calculated 't' values and 'F' values do not exceed theoretical values. It is noticed from the Table 2, that there is no significant difference between the proposed methods and the official method, showing that these methods are as accurate and precise as the official method. Various excipients present in the tablet did not interfere in the proposed method. In conclusion, we suggest that the methods presented here are suitable for the determination of hexamine in pure form and in pharmaceutical formulations.

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TABLE 2: DETERMINATION OF HEXAMINE IN HIPREX.

Assay method	Amount found[a](mg)	't'value[b]	'F'value[c]
Official method	429.97±0.729		
UV/Vis	430.68±1.326	0.8144	3.3136
Fluorimetry	429.5±1.248	0.3753	2.9211
HPLC	429.85±0.688	0.4535	1.1245

True average of hexamine moiety per tablet=428.6 mg S; [a]average of five replicates RSD (%); [b]theoretical student $t_{0.05}$ value (d.o.f=4) =2.776; [c]theoretical student F test value for five determinations=5.05.

REFERENCES

1. Wade, O.L., Eds., In; British National Formulatory, Number 6, The Pharmaceutical press, London, 1983, 206.
2. Milanesi, E., **Arch. Farmacol. Sper.** 1927, 43, 205.
3. Nair, J.B., Delaney, M.F. and Combs, K.J., **Anal. Lett.**, 1983, 16, 711.
4. Koga, M., Shinohara, R. and Akiyama, T., **Anal. Sci.**, 1985, 1, 381
5. Strom, J.J., Grady, Jr., and Won, H., **J. Pharm. Sci.**, 1986, 75, 416.
6. Balasubramanian, M., Thennarasu, S. and Perumal, P.T., **Oriental J. Chem.**, 2002, 18, 207.
7. Long, G.L. and Wineforner, J.D., **Anal. Chem.**, 1983, 55, 712A.

Antiinflammatory Activity of Various Extracts of *Celosia argentea* Linn

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Crude ethanol extract of *Celosia argentea* leaves was successively fractionated with petroleum ether, solvent ether, ethyl acetate, butanol and butanone. The ethanolic extract and various fractions were investigated for antiinflammatory activity in rats at a dose of 100 mg/kg i.p. The ethanolic extract exhibited antiinflammatory activity when compared to control and was comparable to that of standard drug aspirin.

Celosia argentea Linn. (Amaranthaceae) is a small plant, which distributed in India, Sri Lanka, Africa, America and Taiwan. Traditionally the dried plant is used as antiscorbutic[1]. The leaves are antipyretic, reduce inflammations, strengthen the liver and were useful in gonorrhoea and burns. The seeds are bitter and useful in blood diseases and mouth sores[2]. The seeds were reported to be an efficacious remedy in diarrhoea[3].

A literature survey reveals that no scientific study has been reported with respect to antiinflammatory properties of *Celosia argentea* Linn. Therefore, in the present commu-

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