TABLE 3: MELTING POINTS OF THE 2-CHLOROACETANILIDES.

Cmpd.	I: R_1=R_2=CH_3 Y=Cl	M.P.° (range)
Id	R_3 = $COCH_3$	193-194
Ie	= NO_2	207.208
If	= F	203-204

a solution of 1.39 g (5 mmol) of Ij in 15 ml of 10% HCl in an ice-salt bath to ~0°. To this, a solution of 0.38 g (5.5 mmol) $NaNO_2$ in 10 ml water was slowly added. Another 30 ml of ice cold HCl was added 15 min after the addition of $NaNO_2$ solution. The reaction mixture was allowed to warm without the cooling bath. Bubbling was noticed at ~20°. The temperature was slowly raised to 95° by warming in a water bath. The solution was neutralized with a mixture of solid $NaHCO_3$ and saturated $NaHCO_3$ solution. The organic product was extracted with CH_2Cl_2, washed with water and dried over Na_2SO_4. Removal of solvent gave 1.04 g crude product which was purified by column chromatography over 30 g alumina using 90:10 CH_2Cl_2:hexane followed by CH_2Cl_2 and then increasing proportions of isopropyl alcohol in CH_2Cl_2 as the solvents. Fractions that gave residues with m.p. close to each other were combined. The total weight of fractions of m.p. 174-175° (represented 40.3% yield).

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Spectrophotometric Method for the Determination of Tobramycin in Pharmaceutical Formulations

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A sensitive and accurate spectrophotometric method for the quantitative determination of tobramycin in either pure form or in injections is proposed. The method is based on the development of a green coloured product with 3-methyl-2-benzothiazolinone hydrazone hydrochloride and ferric chloride having an absorption maximum at 645 nm. Beer's law is obeyed in the concentration range of 50-500 μg/ml. The optimum reaction conditions and other analytical parameters are statistically evaluated.

Tobramycin (TM) is a simple aminoglycoside antibiotic with an extended spectrum of activity against gram negative and aerobic bacilli[1]. It is official in Indian Pharmaco-

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poeia[2]. It is chemically known as O,3-amino-3-deoxy-α-D-glucopyranosyl[1→6]-O-[2,6-diamino-2,3,6-trideoxy-α-D-ribo-hexoglycopyranosyl]-2-deoxy streptamine[3]. The existing analytical procedures reported for its determination are based mainly on either HPLC[4-5] or conductometric determination[6]. Other methods include liquid chromatography[7] and turbidimetry[8]. The only reported visible spectrophotometric procedure[9] for its determination is based on derivatisation using o-phthaldialdehyde, fluorescamine and dansyl chloride.

In the present paper, a simple, accurate and sensitive spectophotometric method for the determination of TM molecule using methyl benzothiazolinone hydrazone hydrochloride (MBTH) and ferric chloride is described. MBTH when treated with an oxidising agent, undergoes oxidation with loss of two electrons and one proton forming an electrophilic intermediate which by oxidative coupling forms the coloured complex with the drug.

All the chemicals used were of analytical grade. Aqueous solutions of ferric chloride (0.7%) and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (0.2%) (S.D. Fine Chem. Ltd., Mumbai) were prepared in distilled water. The commercial formulations were procured from the local market. Spectral and absorbance measurements were made on an Elico SL 171 mini spectophotometer. Working standard solution was prepared by dissolving 100 mg of TM in 100 ml of distilled water (1 mg/ml).

Aliquots of working standard solution of TM ranging from 0.5 to 5 ml were transferred into a series of 10 ml volumetric flasks and to each volumetric flask, 1.5 ml of MBTH reagent and 2 ml of $FeCl_3$ were added and the contents mixed well. Then the volume of each volumetric flask was adjusted to 10 ml with distilled water and set aside for 20 mm. The absorbance was measured at 645 nm against a reagent blank. The amount of TM present in the sample solution was computed from its calibration curve.

The optical characteristics such as Beer's law limits, Sandell's Sensitivity, percent relative standard deviation and % range of error were calculated for the method and results were summarised in the Table. The method was applied for the analysis of the drug in injections. To evaluate the validity and reproducibility of the method, known amount of the pure drug was added to the previously analysed pharmaceutical formulations and the formulations analysed by the proposed method, the percentage recovery was found to be 99.99. In conclusion, the proposed method is economical, simple, sensitive and accurate enough for the routine determination of tobramycin in bulk as well as in injection form.

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY.

Parameters	Method
λmax	645nm
Beer's law limit (μg/ml)	50 to 500
Molar absorptivity (l/mol.cm)	9.346×10^2
Correlation coefficient	0. 9999
Sandell's sensitivity (μg/cm² absorbance unit/0.01)	0.0467
Regression equation (Y=mx+c)[a]	
Slope (b)	1×10^{-3}
Intercept (a)	1.29
Relative standard deviation	2.184
% range of error[b]	2.123
Confidence limit with 0.01 level	3.550
Confidence limit with 0.05 level	

[a]with respect to Y=mx+c, where c is concentration (μg/ml) and y is absorbance. [b]Eight replicate samples.

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Spectrophotometric Determination of 4,4'-Sulphonyldianiline

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A simple, rapid and sensitive spectrophotometric method for the determination of 4,4'-sulphonyldianiline (dapsone) is described. The method is based on the formation of orange red coloured product by the diazotisation of dapsone followed by complexation with dopamine in presence of molybdate ions in 1:1 sulphuric acid medium. The product is stable for two days at 27°. Beer's law is obeyed in the concentration range of 0.1–8.0 μg/ml at 510 nm. The method is successfully employed for the determination of dapsone in tablets and common excipients used as additives in pharmaceuticals do not interfere. The method offers the advantages of simplicity, rapidity and sensitivity without the need for extraction or heating. Limit of detection and limit of quantification are reported.

4,4'-sulphonyldianiline or dapsone (DAP) is used in the treatment of dermatitis herpetiformis[1]. It is used as an antileprotic agent and also as a non-steroidal antibacterial drug. It is also used as an antiparasitic and a commonly used medication for HIV and AIDS patients. An excellent review of pharmacology and therapeutic use of DAP is given by Uetrecht[2]. DAP is also used as a reagent for the determination of various substances[3-6]. DAP is official in British Pharmcopoeia[7,8] and United States Pharmacopoeia[9]. There are various methods available for the determination of DAP, which include HPLC[10-12], liquid chromatography[13], PMR spectroscopy[14], thermometric titration[15] and spectrophotometry[16-22]. The spectrophotometric methods, which have already been reported, suffer from lack of sensitivity, involvement of heating or extraction, longer time taken for completion of reaction and narrow detection limit.

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In the present work, the diazotised DAP is made to react with dopamine hydrochloride (DPH) followed by the addition of sodium molybdate in presence of 1:1 sulphuric acid medium to give an orange red product. This colour reaction is being reported for the first time.

A Jasco Model Uvidec-610 UV/Vis spectrophotometer with 1.0 cm matched cells was used for absorbance measurements. Both DAP and DPH were purchased from Sigma Chemical Co., St. Louis, MO, USA. Molybdic acid was purchased from Merck, Germany and BDH sample of sodium nitrite was used. AR sulphuric acid was used for the experiment. All other reagents and solvents were of analytical grade. Commercial dosage forms were purchased from Burroughs Wellcome.

Deionized water was used to prepare all solutions. Standard solution of DAP (1000 μg/ml) was prepared by dissolving 100 mg of DAP in 2-3 ml of 1.0 M sulphuric acid and