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Simultaneous determination of methylene blue, hexamethylene tetramine and resorcinol in pharmaceutical formulations by first-derivative UV spectrophotometry

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Summary

In this paper, rapid and precise first-derivative UV spectrophotometric assay procedures are described for the simultaneous determination of methylene blue, hexamethylene tetramine and resorcinol in their binary mixtures. The methods have been applied successfully to a dragee containing methylene blue-hexamethylene tetramine and a mouth-wash formulation containing methylene blue-resorcinol combinations. The relative standard deviation was found to be 0.71% for methylene blue at 273.0 nm and 0.70% for hexamethylene tetramine at 221.3 nm in the first combination and 0.21% for methylene blue at 300.0 nm and 0.54% for resorcinol at 260.4 nm in the second combination.

A methylene blue-hexamethylene teramine combination is used as a urinary antiseptic and a methylene blue-resorcinol combination as an antiseptic and fungistatic for the oral cavity. Published reports on mixtures containing methylene blue are scarce in the literature. Methylene blue, novocaine, quinine · HCl and analgin were simultaneously determined in their binary mixtures containing methylene blue by potentiometric titration with NaOH after separation by using ion-exchange chromatography on cationic KU 2 (Koka, 1977).

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This paper describes the use of first-derivative spectrophotometry for the simultaneous analysis of methylene blue-hexamethylene tetramine combinations in dragees and methylene blue-resorcinol combinations in mouth-wash preparations simply, precisely and without preliminary separation procedures.

In this work, methylene blue and hexamethylene tetramine were obtained from Drifen Pharmaceutical Industries, and resorcinol was purchased from Egas Pharmaceutical Industries. Hydrochloric acid was analytical reagent grade from (Merck Ltd.).

Measurements were made using a Shimadzu UV-160 double-beam UV-visible spectrophotometer with a fixed slit width (3 nm). The first-

derivative curves of the UV spectra of reference and test solutions were recorded in 1-cm quartz cells over the range 200–350 nm ($\Delta\lambda = 2$ nm) by using its own recorder. The scan speed was 60 nm/s: the ordinate minimum and maximum settings were 0.6 and −0.2 for the methylene blue-resorcinol mixture and 0.3 and −0.4 for methylene blue-hexamethylene tetramine mixture.

As standard solutions; an accurately weighed 100 mg quantity of methylene blue reference standard was dissolved separately in 100 ml of 0.1 N HCl and water; 100 mg of hexamethylene tetramine was dissolved in 0.1 N HCl and 100 mg of resorcinol was dissolved in 100 ml water. These solutions were used in the construction of calibration curves and for spectra after appropriate dilution with the same solvents.

By employing these methods, two commercial preparations were assayed. Their declared contents were as follows:

Dragee (proprietary name: Hemo-blue)
methylene blue: 40 mg
hexamethylene tetramine: 500 mg/dragee

Mouth-wash preparation (proprietary name: Buco-blue)
methylene blue: 150 mg
resorcinol: 75 mg/15 ml

Preparation of samples was as follows. (a) Dragee – 20 dragees were accurately weighed and powdered in a mortar. An amount of the dragee mass equivalent to the content of one dragee was dissolved in 60 ml of 0.1 N HCl. After 30 min of mechanical shaking, the solution was filtered through Whatman no. 42 filter paper in a 100 ml graduated flask. The residue was washed three times with 10 ml of solvent, and the volume was then made up to 100 ml with the same solvent. This solution was diluted 1:250 with 0.1 N HCl. (b) Mouth-wash preparation – 1 ml of formulation was pipetted in a 100 ml graduated flask and the volume was made up to 100 ml with water. This solution was then diluted 1:10 with water.

For the analysis of the results, the $dA/d\lambda$ values were read in the first-derivative spectra of the standard and sample solutions and hence, in the sample, they were calculated from the proportional relationship that exists between the measured $dA/d\lambda$ value and concentration.

Methylene blue is a colored compound (λ_{max} in 0.1 N HCl and water: 664.2 nm) but its co-existing compounds, hexamethylene tetramine (**I**) and resorcinol (**II**) are colorless in their solutions. Therefore, methylene blue can readily be determined in the presence of these compounds without prior separation. However, for **I** and **II**, quantitation is less simple in the presence of methylene blue, due to spectral overlap in the UV region. Nevertheless, in the first derivative spectra of methylene blue-hexamethylene tetramine and methylene blue-resorcinol mixtures over the same wavelength interval, their simultaneous determination becomes possible (Figs 1 and 2).

(a) Methylene blue-hexamethylene tetramine mixture. Simultaneous determination of methylene blue and hexamethylene tetramine was achieved simply by reading the first-derivative absorbances ($dA/d\lambda$) of the solution of the mixture in 0.1 N HCl at 273.0 and 221.3 nm, respectively (Fig. 1) based on the fact that co-formulated substances have zero-derivative absorbance in these wavelengths. This indicates that no interference arises from the co-existing substances at these wavelengths. For methylene blue, 273 nm in the first-derivative spectra was selected instead of 664.2 nm in the zero-order absorption spectra for two reasons: first, because of its simplicity, recording of the absorbance values in this manner for the two compounds in the same spec-

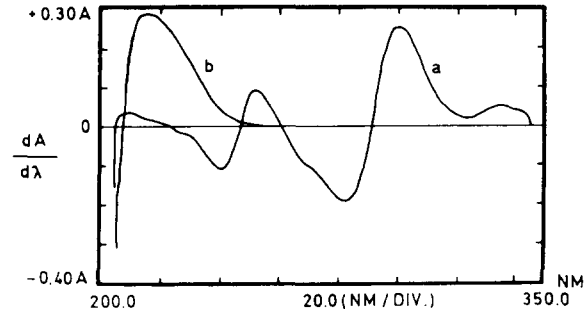

Fig. 1. First-derivative spectra of (a) 20.5 μg/ml solution of methylene blue, and (b) 6.0 μg/ml solution of hexamethylene tetramine in 0.1 N HCl.

tra was achieved and the results were satisfactory; second, the intensity of the band at 664.2 nm was very high, and hence further dilution was necessary, however, this dilution caused a decrease in the value of the derivative absorbance for hexamethylene tetramine and the sensitivity was reduced.

In this method, the relative standard deviation was found to be 0.71% for methylene blue and 0.70% for hexamethylene tetramine in the corresponding synthetic mixtures prepared by adding known amounts of these active ingredients. The regression equations were $Y = 1.1 \times 10^{-1}X - 2.5 \times 10^{-2}$ for methylene blue and $Y = 1.4 \times 10^{-2}X - 4.4 \times 10^{-3}$ for hexamethylene tetramine (where Y is the derivative absorbance and X denotes the concentration in μg/ml) for the $dA/d\lambda$ values at 273.0 and 221.3 nm, respectively, in the first-derivative spectra of 10 solutions each in 0.1 N HCl. The correlation coefficients of the calibration curves were 0.9996 and 0.9997, respectively. The concentration range for compliance with Beer's law was 1.2–6.1 μg/ml for methylene blue and 1.5–20.6 μg/ml for hexamethylene tetramine.

(b) Methylene blue-resorcinol mixture. Simultaneous determination of methylene blue and resorcinol was achieved simply by noting the respective $dA/d\lambda$ values of the aqueous solution of the mixture at 300.0 nm (Fig. 2). Using this procedure, the relative standard deviation was found to be 0.21% for methylene blue and 0.54% for resorcinol in the synthetic mixtures prepared via the addition of known amounts of these active compounds. The regression equations were $Y = 2.4 \times 10^{-2}X - 1.8 \times 10^{-2}$ for methylene blue and $Y = 7.0 \times 10^{-2}X + 2.0 \times 10^{-1}$ for resorcinol. The corresponding correlation coefficients of the calibration curves were 0.9998 and 0.9993. The concentration range for compliance with Beer's law was 6.0–22.4 μg/ml for methylene blue and 3.0–12.5 μg/ml for resorcinol.

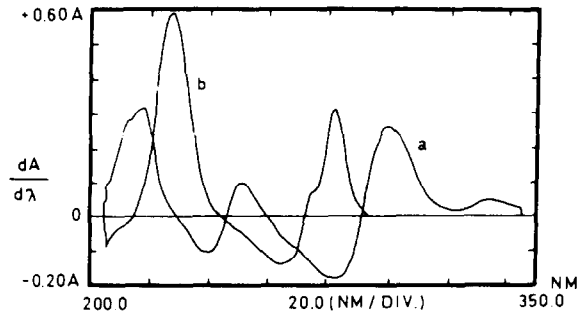

Fig. 2. First-derivative spectra of (a) 20.5 μg/ml solution of methylene blue, and (b) 11.2 μg/ml solution of resorcinol in water.

Spectrophotometric recordings showed no interference from the excipients (starch, talc, magnesium stearate and, pharmaceutical dyes and sugars used in the coating material of the dragee preparation).

In the assay of pharmaceutical preparations, the declared amount of active ingredients as a percentage ± S.D. was found to be 99.7 ± 0.4 for methylene blue and 98.8 ± 0.4 for hexamethylene tetramine in the dragee and 99.7 ± 0.4 for methylene blue and 99.5 ± 0.3 for resorcinol in the mouth-wash preparation (results are the average of 10 experiments in each case).

These methods were found to be suitable for the routine analysis of commercial formulations by virtue of their simplicity and reliability.

References

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