Drug Development and Industrial Pharmacy
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/iddi20

Analysis of Diphenhydramine Hydrochloride and Naphazoline Hydrochloride in Presence of Methylene Blue in Eye Drops by Second Derivative Spectrophotometry
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Published online: 12 Apr 2015.

To cite this article: M. A. Korany, Mona M. Bedair & A. El-Gindy (1990) Analysis of Diphenhydramine Hydrochloride and Naphazoline Hydrochloride in Presence of Methylene Blue in Eye Drops by Second Derivative Spectrophotometry, Drug Development and Industrial Pharmacy, 16:9, 1555-1564
To link to this article: http://dx.doi.org/10.3109/03639049009074383

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ANALYSIS OF DIPHENHYDRAMINE HYDROCHLORIDE AND NAPHAZOLINE HYDROCHLORIDE IN PRESENCE OF METHYLENE BLUE IN EYE DROPS BY SECOND DERIVATIVE SPECTROPHOTOMETRY.

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** Control Laboratory, The Alexandria Co. for Pharmaceutical and Chemical Industries.

ABSTRACT
A second (D₂) derivative spectrophotometric technique has been applied for the determination of diphenhydramine hydrochloride in mixture with naphazoline hydrochloride and in presence of Methylene blue. Diphenhydramine hydrochloride has been extracted with chloroform and D₂-value was measured at 255 nm. Naphazoline hydrochloride in the mixture has been determined by direct measurement of its D₂ at 273 nm. Methylene blue could be determined in the mixture by direct absorbance measurement at 663 nm. As illustrate example, commercial eye drops was analysed for these two drugs in presence of methylene blue in pharmaceutical preparation. The results obtained were of high accuracy and good reproducibility.

INTRODUCTION
The antihistamin diphenhydramine hydrochloride has been dispensed in several galenical formulations. Diphenhydramine hydrochloride has been determined by several spectrophotometric¹,²

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and colorimetric\textsuperscript{3} methods of analysis. Direct and indirect non-aqueous titration methods have been also applied\textsuperscript{4,5}. The official method use the non-aqueous titration for diphenhydramine assay\textsuperscript{6}. The chromatographic techniques of analysis, TLC\textsuperscript{7}, GLC\textsuperscript{8,9} and HPLC\textsuperscript{10} have been lately employed. Application of orthogonal function (equal and non-equal) for diphenhydramine assay in single\textsuperscript{11} and in two-component mixture\textsuperscript{12} have been reported. Use of computer aided second derivative multi-channel UV spectrophotometry was described\textsuperscript{13} for diphenhydramine assay.

Naphazoline salt has been determined colorimetrically after treatment with different chromogenic reagents\textsuperscript{14,15} chromatographic techniques including TLC\textsuperscript{16}, and GLC\textsuperscript{17} have been reported. Mixture of naphazoline-antazoline has been studied by many authors\textsuperscript{18,19}, while mixture of diphenhydramine-naphazoline in eye drops solution have been also studied using non-aqueous titrations\textsuperscript{4} and GLC\textsuperscript{8}.

Only few reports of the use of derivative spectrophotometry has been published for the assay of two component\textsuperscript{20-23} and three component mixtures\textsuperscript{24}. Determination of certain drugs in the presence of their degradation products are also reported\textsuperscript{25-27}.

This work deals with the second derivative spectrophotometric determination of mixture of diphenhydramine and naphazoline. The commercial (Occumethyl) eye drops\textsuperscript{6} was chosen as an example of application of the proposed method in pharmaceutical preparation.

**MATERIALS AND METHODS**

**Materials:**

All drugs, chemicals and solvents were analytical grade.

**Apparatus:**

The Perkin-Elmer Model 551S UV-VIS and Hitach Model 561 recorder were used. The ($D_2$) curves were recorded in 1 cm quartz cell using: scan speed, 120 nm min\textsuperscript{-1}; chart speed 120 nm min\textsuperscript{-1}; spectralslit with, 2; recorder range, IV; response time, 2 s.
(for diphenhydramine hydrochloride) and 4 s (for naphazoline hydrochloride). The minimum and maximum amplitude of the most concentrated standard solution were adjusted at not less than 80% of the recorder full-scale deflection (25 cm).

**Preparation of different standard solutions:**
(a) A solution of 250 μg/ml of diphenhydramine HCl was accurately prepared in 1 N hydrochloric acid.
(b) A solution of 10 μg/ml of naphazoline hydrochloride was accurately prepared in 0.01 N hydrochloric acid.
(c) A solution of 3 μg/ml of methylene blue was accurately prepared in 0.1 N sulphuric acid.

**Sample preparation**
(a) For diphenhydramine hydrochloride: A volume of 25.00 ml of eye drops solution was diluted to 100.00 ml with 1 N hydrochloric acid.
(b) For naphazoline hydrochloride: A volume of 10.00 ml of the eye drops solution was diluted to 100.00 ml with water.
(c) For methylene blue: A volume of 10.00 ml of the drops solution was diluted to 100.00 ml with 0.1 N sulphuric acid.

**General procedure**
(a) For diphenhydramine hydrochloride: A volume of 10.00 ml of each of the prepared standard (methylene blue predetermined amount according to analysis of the sample was added) or sample solution was transferred into separating funnel. A 5 ml saturated sodium chloride solution was added to each separating funnel, and diphenhydramine hydrochloride was extracted with two successive 12 ml portions of chloroform, collecting them into 25-ml volumetric flask and completing the volume with chloroform. The D₂ curves were recorded for both extracts against chloroform blank. The peak-tangent at 255 nm for each curve was measured in mm (Figure 1b).
(b) For naphazoline hydrochloride: A volume of 10.00 ml of the prepared sample (or appropriate volume of standard solution) was transferred into 100 ml volumetric flask, completing the volume with 0.01 N hydrochloric acid. The D₂ curves were recorded against
(a) Zero-order absorption spectra and (b) second derivative spectra of 100 µg/ml of each of diphenhydramine hydrochloride (—) and naphazoline hydrochloride (---) and 3 µg/ml of methylene blue (...) in chloroform after applying the extraction procedure.

0.01 N hydrochloric acid blank. The peak height at 273 nm for each curve was measured in mm (Figure 2b).

(c) For methylene blue: The absorbance ($A_{max}$) of the prepared sample (or appropriate volume of standard solution) was measured at 663 nm using 0.1 N sulphuric acid blank.
FIGURE 2

(a) Zero-order absorption spectra and (b) second derivative spectra of 10 µg/ml of each of diphenhydramine hydrochloride (---) and naphazoline hydrochloride (----) and 0.3 µg/ml methylene blue (---) in 0.01 N hydrochloric acid.

RESULTS AND DISCUSSION

Due to the large difference in the $A_{1	ext{ cm}}^{\%}$ values for diphenhydramine hydrochloride (I) (13.3 at 258 nm) and naphazoline hydrochloride (II) (253 at 280 nm) in 0.1 N hydrochloric acid, the former might be extracted from the mixture with chloroform in the presence of sodium chloride. Such extraction procedure was based on the
### Table 1

**Recovery of Diphenhydramine Hydrochloride and Naphazoline Hydrochloride Added to Commercial Eye Drops.**

<table>
<thead>
<tr>
<th>No. of Exp.</th>
<th>Diphenhydramine Hydrochloride Added ug/ml Chloroform</th>
<th>Naphazoline Hydrochloride Added ug/ml 0.01 N HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labelled Recovery, %</td>
<td>Labelled Recovery, %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100.32</td>
<td>100.00</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100.32</td>
<td>100.79</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>99.56</td>
<td>99.29</td>
</tr>
<tr>
<td></td>
<td>Mean 100.03</td>
<td>Mean 100.03</td>
</tr>
<tr>
<td></td>
<td>±S.D. 0.28</td>
<td>±S.D. 0.69</td>
</tr>
</tbody>
</table>

* Donated by Alexandria Co. for Pharmaceutical and Chemical Industries, Alexandria, Egypt and complied with compendial requirements.

** Occumethyl eye drops: labelled to contain 10 mg diphenhydramine hydrochloride, 10 mg naphazoline hydrochloride, 0.3 mg methylene blue, 10 mg zinc sulphate and sodium citrate, citric acid, benzalkonium chloride, sodium chloride, hydroxy propylmethyl cellulose as adjuvants per 10 ml drops.

Differential solubility of I in chloroform which II was very slightly soluble in. After extraction, the remaining contribution of II can be corrected for by applying the D2 measurement at 255 nm of the mixture (Figure 1b). Methylene blue (III) the minor component in the eye drops mixture exhibits small interference on assaying I by the proposed method. To correct for such error, precalculated amount of III similar to that in the sample must be added to reference standard of I.

Figure 1a shows the zero order UV spectra of I, II and III in chloroform after applying the extraction procedure and in similar
### TABLE 2

**ASSAY RESULTS FOR THE DETERMINATION OF DIPHENHYDRAMINE HYDROCHLORIDE AND NAPHAZOLINE HYDROCHLORIDE IN LABORATORY-MADE MIXTURE AND IN COMMERCIAL EYE DROPS.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent Recovery&lt;sup&gt;a&lt;/sup&gt; (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assays Diphenhydramine hydrochloride</td>
</tr>
<tr>
<td></td>
<td>D&lt;sub&gt;2&lt;/sub&gt;-Method 3-Component Method</td>
</tr>
<tr>
<td>Laboratory-made Mixture&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 100.26±0.58 93.86±1.39 99.61±0.43 98.97±0.40</td>
</tr>
<tr>
<td></td>
<td>P= 5.88 1.16 (6.39)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eye-Drops</td>
<td>6 99.8±0.71 101.13±4.62 100.09±0.48 102.03±0.70</td>
</tr>
</tbody>
</table>

<sup>a</sup> Recovery from nominal content.

<sup>b</sup> Concentration range 80-122 µg per ml chloroform (for both drugs) in determination of diphenhydramine hydrochloride in the mixture, and 8-12 µg per ml in 0.01 N hydrochloric acid for both drugs in the determination of naphazoline hydrochloride in the mixture.

<sup>c</sup> Precalculated amount of methylene blue were added to each synthetic mixture to give the same ratio as in commercial eye-drops (1:1:0.03).

Values in parenthesis are the theoretical values at P=0.95.

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ratio like the eye drops. Figure 1b shows their corresponding D<sub>2</sub> curves. From that figure it was clear that I can be determined by measuring the peak-tangent at 255 nm. Meanwhile, the direct measurement of D<sub>2</sub> at 255 nm as a method for determination of I in mixture with II in eye drops solution can not be accepted.

Figure 2a shows the UV spectra of the same mixture in 0.01 N hydrochloric acid without applying the extraction process, while Figure 2b shows their corresponding D<sub>2</sub> curves. Accordingly, II can be assayed in the mixture by direct D<sub>2</sub> measurement at 273 nm.
Under the described experimental conditions, the graphs obtained by plotting $D_2$ value versus concentration, C, show linear relationships. The concentration range following these relationships were 40-100 µg/ml for I and 5-15 µg/ml for II. The three linear regression equations were found to be,

$$D_2 = -1.8400 + 6.3000 C \quad \text{(I with III)}$$
$$D_2 = 0.0321 + 0.5446 C \quad \text{(I without III)}$$
$$D_2 = 0.3255 + 4.2016 C \quad \text{(II)}.$$

In which $C$ is the concentration in µg/ml. The correlation coefficients were 0.9997, 0.9993 and 0.9999 with standard deviation of ±0.23, ±0.28 and ±0.24, respectively (using 6 separate determinations).

Recovery experiments (Table 1) have been carried out using different known concentrations of I or II added to the final eye drops solutions (standard addition method).

The proposed method has further been applied to the determination of both drugs in commercial eye drops and in laboratory-made mixtures prepared in different proportions of drug components (Table 2). The proposed method was compared with the three component method (manufacturer's results). Subjecting the results of synthetic mixture for statistical analysis the $D_2$ method gave generally more accurate (t-test) and equal reproducible (F-test) results compared with the three-component method.

Different batches of the commercial eye drops were assayed for both I and II with mean percentage found ± SD equal to 99.31 ± 1.63, 99.51 ± 2.02, respectively.

In conclusion, using the $D_2$ method a weakly absorbing compound (diphenhydramine hydrochloride) can be assayed with almost equal level of accuracy to that of strongly absorbing compound (naphazoline hydrochloride). Methylene blue can be easily determined by measuring its blue colour at 663 nm with apparent $A_1^\%$ cm 1975.
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