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Colorimetric methods for the assay of nomifensine maleate*

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Abstract: Two colorimetric methods are reported for the assay of nomifensine maleate. The methods are based on coupling between the diazotised form of nomifensine maleate and (i) *N*-(1-naphthyl)-ethylene diamine dihydrochloride (Bratton–Marshall reagent) and (ii) *p*-aminosalicylic acid (PAS). The optimum conditions for the reactions were investigated. The coupled products exhibit maximum absorbance at 470 and 435 nm for the Bratton–Marshall and PAS reagents, respectively. With PAS, a linear relationship has been established between absorbance (A_{max}) and concentration of nomefensine maleate over the range 2–12 μg ml^{-1}. Similarly, with the Bratton–Marshall reagent, a linear relationship exists in the concentration range 2–16 μg ml^{-1}. The calculated mean percent recoveries for nomifensine maleate in the commercial capsules (Merital® 25 mg) using the Bratton–Marshall and PAS reagents were 97.53 ± 0.71 and 100.28 ± 1.32, respectively. Similarly, for the added recoveries, the percentages obtained were 99.01 ± 0.46 and 100.03 ± 1.03, respectively.

Keywords: *Nomifensine maleate; colorimetric determination; diazotisation; coupling with* p-*aminosalicylic acid; Bratton–Marshall reagents; pharmaceutical analysis.*

Introduction

Nomifensine (8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline) maleate is a psychotropic agent used for the treatment of depression. A number of methods have been reported for the drug including polarography and amperometry [1], radioimmunoassay [2], thin layer chromatography [3], gas–liquid chromatography [4, 5] and high-performance liquid chromatography [6]. Most of these methods dealt mainly with the determination of nomifensine in the presence of its metabolites in biological fluids. In view of the fact that nomifensine possesses a primary aromatic amino group, it was deemed useful in developing sensitive colorimetric methods for its determination in capsule dosage forms.

The introduction of *N*-(1-naphthyl)-ethylene diamine dihydrochloride [7, 8] (Bratton–Marshall reagent) *p*-aminosalicylic acid and (PAS) [9] have led to numerous

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applications as analytical reagents. They have been applied to the colorimetric determination of aromatic amino compounds. The Bratton–Marshall reagent was particularly useful for the colorimetric determination of sulphonamides [7]. The results obtained by the two proposed methods are most encouraging with regard to sensitivity, precision and accuracy. These methods are simple, and can be used for the quality control assay of the drug.

Experimental

Apparatus, materials and reagents

A Varian DMS 90 double-beam spectrophotometer with 1-cm quartz cuvettes was used. Nomifensine maleate standard solution was prepared fresh daily by dissolving 100 mg of nomifensine maleate powder (Hoechst, W. Germany) in 100 ml 0.5 M phosphoric acid. Nomifensine maleate commercial capsules (Merital® 25 mg) were gifts from Hoechst, W. Germany. Sodium nitrite solution was prepared fresh daily by dissolving 100 mg of sodium nitrite (BDH, Poole, UK, Analar) in 100 ml distilled water. *N*-(1-naphthyl)-ethylene diamine dihydrochloride solution was prepared by dissolving 100 mg of the reagent (BDH, Poole, UK, Analar) in 100 ml distilled water. *p*-Aminosalicylic acid solution was prepared by dissolving 200 mg of the sodium salt (BDH, Poole, UK, Analar) in 100 ml distilled water.

(i) *Procedure using the Bratton–Marshall reagent*

Portions of 0.1–0.8 ml nomifensine maleate standard solution were accurately transferred into eight 50-ml volumetric flasks. 0.5 ml sodium nitrite solution was added. They were mixed thoroughly and allowed to stand for 2 min at room temperature (20°C). 1 ml of methanol was added, followed by 3 ml of Bratton–Marshall reagent, and the solution was allowed to stand for 30 min.

It was then diluted to volume with distilled water and the absorbance was measured at 470 nm against a blank solution which was prepared simultaneously. Absorbance was plotted against concentration of nomifensine maleate to establish the standard curve, or the equivalent regression line equation was calculated.

(ii) *Procedure using PAS reagent*

Portions of nomifensine maleate standard solution (0.1–0.6 ml) were pipetted into six 50-ml volumetric flasks. 3 ml *p*-aminosalicylate salt solution was added followed by 0.5 ml sodium nitrite solution. The solution was allowed to stand for 60 min at room temperature. It was diluted to volume with distilled water, and then the absorbance was measured at 435 nm using an appropriate blank prepared simultaneously. Absorbance was plotted against concentration of nomifensine maleate to establish the calibration curve or the equivalent regression line equation was calculated.

Procedure for sample

The contents of 10 capsules were accurately weighed and the mean value of the weight of capsule contents was calculated. An amount of powder equivalent to about 25 mg nomifensine maleate was accurately weighed and transferred into a 25-ml volumetric flask. 20 ml of 0.5 M phosphoric acid were added and the solution was shaken for 10 min. It was then diluted to volume with 0.5 M phosphoric acid and filtered.

The following procedures were applied for the sample filtrate:

(a) *Bratton–Marshall reagent method.* Portions of 0.1–0.8 ml of the filtrate were pipetted into 50-ml volumetric flasks. The procedure was continued as described for the standard solutions using Bratton–Marshall reagent. [See section (i), from "0.5 ml sodium nitrite solution was added".] From the prepared calibration curves the concentration of nomifensine maleate in the sample was determined. Alternatively, the concentration can be calculated from the regression equation.

(b) p-*Aminosalicylic acid method.* Portions (0.1–0.6 ml) of the sample filtrate were pipetted into 50 ml volumetric flasks. The procedure was continued as described for the standard solutions. [See section (ii), from "3 ml *p*-aminosalicylate salt solution".] From the prepared calibration curve the concentration of nomifensine maleate in the sample was determined. Alternatively, the concentration can be calculated from the regression equation.

Results and Discussion

The diazotised form of nomifensine maleate couples with *N*-(1-naphthyl)-ethylene diamine and *p*-aminosalicylic acid to yield red and yellow azo dye derivatives with absorbance maxima at 470 and 435 nm, respectively (Figs 1 and 2). The reactions of *N*-

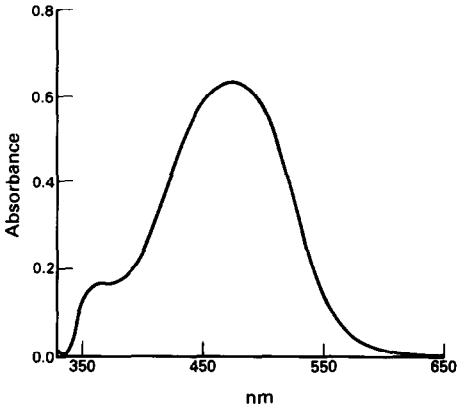

Figure 1
Absorption spectrum of the diazotised form of nomifensine maleate and *N*-(1-naphthyl)-ethylene diamine dihydrochloride.

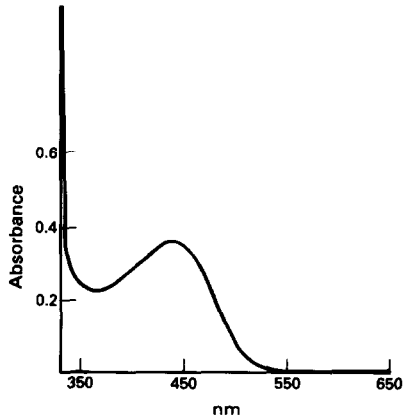

Figure 2
Absorption spectrum of the diazotised form of nomifensine maleate and *p*-aminosalicylic acid.

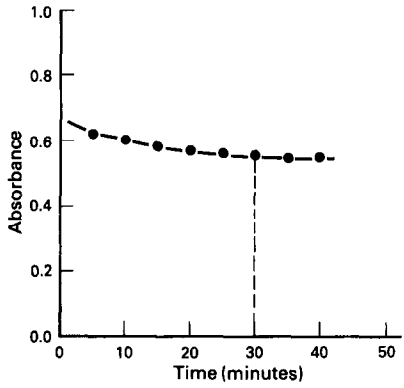

Figure 3
The relationship between time and absorbance for completion of the diazotisation and coupling reaction between diazotised nomifensine maleate and *N*-(1-naphthyl)-ethylene diamine dihydrochloride.

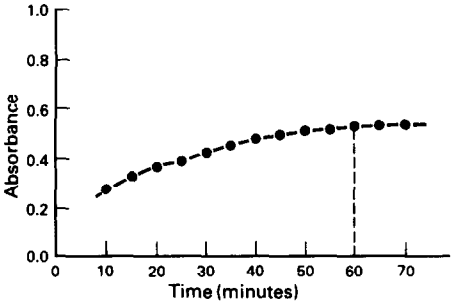

Figure 4
The relationship between time and absorbance for completion of the diazotisation and coupling reaction between diazotised nomifensine maleate and *p*-aminosalicylic acid.

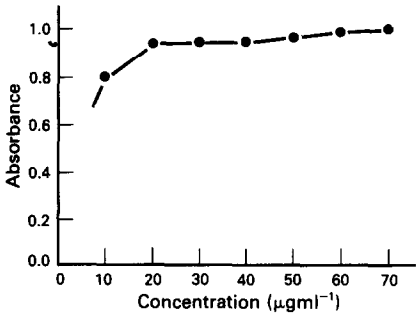

Figure 5
Optimum concentration for *N*-(1-naphthyl)-ethylene diamine dihydrochloride required for maximum colour development.

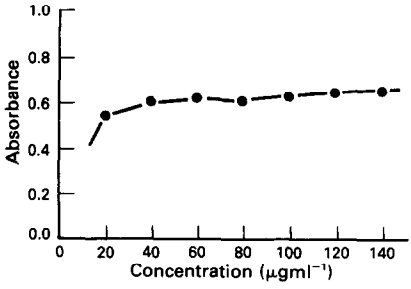

Figure 6
Optimum concentration for *p*-aminosalicylic acid required for maximum colour development.

Table 1
Assay of nomifensine maleate capsules by the two proposed methods

Bratton–Marshall reagent method					*p*-Aminosalicylic acid (PAS) method				
Claimed weight of drug in sample weighed (mg)	% Found	Claimed weight of drug in sample weighed (mg)	Weight of authentic drug added (mg)	% Recovery	Claimed weight of drug in sample weighed (mg)	% Found	Claimed weight of drug in sample weighed (mg)	Weight of authentic drug added (mg)	% Recovery
25.0	96.4	10	10	98.5	25	98.5	10	10	99.4
24.8	98.2	15	10	99.6	24.8	100.4	15	10	100.1
25.1	97.3	10	15	99.1	25.1	99.3	10	15	98.2
25.4	98.4	12.5	12.5	99.5	25.4	102.0	12.5	12.5	101.5
25.2	97.4	11.5	13.5	98.6	25.2	101.5	11.5	13.5	101.0
25.0	97.5	13	12	98.8	25.0	100.0	13	12.0	99.5
Mean ± S.D. = 97.53 ± 0.71		Mean ± S.D. = 100.28 ± 0.46			Mean ± S.D. = 100.28 ± 1.32		Mean ± S.D. = 100.03 ± 1.03		
F- and *t*-tests for sample only					*F*- and *t*-tests for added recovery				
$F_{0.05}$	3.45 (5.1)*				$F_{0.05}$	5.0 (5.1)*			
$t_{0.05}$	8.98 (2.23)*				$t_{0.05}$	2.19 (2.23)*			

* Significant levels.

(1-naphthyl)-ethylene diamine and *p*-aminosalicylic acid with diazotised aromatic amines are well known azo dye-forming reactions.

The diazotisation and coupling reactions were carried out at 5°C and at room temperature. The most satisfactory results were obtained at ambient conditions. Although the addition of an alkali such as sodium hydroxide, sodium carbonate or sodium acetate to optimise the coupling reaction conditions is a general practice, such additions were found unnecessary since the intensity of colours developed and absorption maxima were not affected when the medium was made alkaline. The minimum times necessary for completion of the diazotisation and coupling reactions are 30 and 60 min for *N*-(1-naphthyl) ethylene diamine dihydrochloride and *p*-aminosalicylic acid reagents, respectively (Figs 3 and 4). The optimum concentrations of both reagents required for maximum colour development are shown in Figs 5 and 6. Under the experimental conditions established using the Bratton–Marshall reagent, the absorbance in 1-cm cells was linearly related to concentration (C, $\mu g\ ml^{-1}$) of nomifensine maleate over the range 2–16 $\mu g\ ml^{-1}$. The regression line equation being $A = 0.016C + 0.005$, and the correlation coefficient, r, is 0.999.

Similarly, when using *p*-aminosalicylic acid reagent, the linear relationship between absorbance and concentration was demonstrated over the range 2–12 $\mu g\ ml^{-1}$. The regression equation was calculated to be $A = 0.054C + 0.001$, and the correlation coefficient, r, is 0.9995.

When the two proposed methods were applied for the analysis of commercial capsules (Merital®-25 mg), the mean percent recoveries obtained were 97.53 ± 0.71 and 100.28 ± 1.32 using the Bratton–Marshall and PAS reagents, respectively.

The results found by applying the two methods to the analysis of nomifensine capsules have been statistically compared (Table 1), with regard to precision and accuracy. Based on the *F*-test at 95% confidence level, there is no significant difference between the precision of two methods. However, by the *t*-test, there is a significant difference between the two means. A set of experiments to determine the added recoveries (Table 1) gave percentages 99.01 ± 0.46 and 100.03 ± 1.03 for the Bratton–Marshall and PAS reagents, respectively. Based on the *F*- and *t*-tests of the added percentage recoveries, the two proposed methods do not differ significantly with regard to precision and accuracy.

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References

[1] K. Nikolic, L. Arsenjevic and M. Medenica, *Acta Pharm. Jugosol.* **34**, 69–74 (1984).
[2] W. Heptner, M.J. Badian, S. Bandner, O.E. Christ, H.M. Fraser, W. Rupp, K.E. Weimer and H. Wissman, *Br. J. Clin. Pharmac.* **4**, Suppl. 2, 123S–127S (1977).
[3] E. J. Baltova and A. Shishkov, *Folia Medica* **25**, 36–41 (1983).
[4] J. Chamberlain and H. M. Hill, *Br. J. Clin. Pharmac.* **4**, Suppl. 2, 117S–121S (1977).
[5] M. P. Quaglio and A. M. Bellini, *Il. Farmaco Ed. Prat.* **39**, 222–232 (1984).
[6] R. L. P. Lindberg, J. S. Salonen and E. I. Iisalo, *J. Chromatogr.* **276**, 85–92 (1983).
[7] A. C. Bratton and E. K. Marshall, *J. Biol. Chem.* **128**, 537–550 (1939).
[8] J. W. Daniel, *Analyst* **86**, 640–643 (1961).
[9] M. Perez, *Bull. Soc. Chim. Biol.* **33**, 195–201 (1951).

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