التقدير الطيفي للاوكسيميتازولين هيدروكلوريد بشكله الحر وفي مستحضراته الصيدلانية باستخدام تفاعلات الأزوتة والاقتران

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الخلاصة
يشمل هذا البحث اقتراح طريقة طيفية دقيقة وبسيطة لتقدير الاوكسيميتازولين هيدروكلوريد في المحول المائي تعتمد الطريقة على تفاعل الاوكسيميتازولين هيدروكلوريد مع الكاشف المؤثر حامض السلفانيلك بوجود هيدروكسيد الصوديوم، لتكوين صبغة أزوتية برتقالية ذاتية في الماء ومستقرة وتعطي أعلى امتصاص عند الطول الموجي 496 نانومتر. كانت حدود قانون بير في مدى 20 - 400 ميكروغرام اوكسيميتازولين في حجم نهائي 25 ملتر (0.8-16جزء/ملليون)، وكان معامل الامتصاص المؤثرية 4.30 بـ 2.30 لتر. مول⁻¹. سم⁻¹. 1% الخطأ النسبي بين 0.28- و97% +، والانحراف القياسي النسبي في مدى 0.27 ± 1.57% اعتدالاً على مستوى التكبير. وتم تطبيق الطريقة بنجاح في تقدير الاوكسيميتازولين في مستحضرات دونليين.

الكلمات المفتاحية: التقدير الطيفي، الأزوتة والاقتران، الاوكسيميتازولين هيدروكلوريد، حامض السلفانيلك.
Spectrophotometric Determination of Oxymetazoline Hydrochloride in Pure and Pharmaceutical Preparations Using Diazo-coupling Reaction

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ABSTRACT

An accurate and simple spectrophotometric method has been suggested for the determination of oxymetazoline hydrochloride (OMCl) in aqueous solution. The present process included coupling of OMCl with diazotized sulphanilic acid reagent in the presence of sodium hydroxide. The orange coloredazo dye formed is very stable and soluble in water and gives maximum absorption at 496 nm. The linearity is obeyed over the range 20 – 400 µg / 25 ml (0.8 – 16 µg.ml⁻¹) the molar absorptivity is equal to 2.30 × 10⁴ L.mol⁻¹.cm⁻¹. The proposed method has been used to determine OMCl in two formulations with satisfactory results.

Keywords: Spectrophotometric determination, Diazotization, Oxymetazoline hydrochloride, Sulphanilic acid.

Introduction

The hydrochloride salt of oxymetazoline is 3-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethyl-phenol hydrochloride(OMCl) [1], it is a sympathomimetic agent with marked α-adrenergic activity has been introduced in some nasal solutions [2]. OMCl is applied to treat epistaxis and eye redness according to minor irritation [3,4].

The large doses of OMCl may cause hypotension, presumably because of a central clonidine-like effect[5].

Various procedures have been illustrated in literature for the determination is: titrimetric method[6], ion selective membrane electrode[7], chromatography[8-10] and flow injection[11]. Also, spectroscopy techniques have been used for determination of OMCl as pure and in various formulations different reagents such as 2,6-dichloroquinone-chlorimide[12] 2,4,6-tris(2-pyridyl)-5-triazine[13], 1,10-phenanthroline[14], 3,5-dinitrosalicylic acid [15], and 4-aminoantipyrine [16].

Our aim is to evaluate a simple spectrophotometric method for the determination of OMCl as pure and in pharmaceutical drop formulations included coupling with diazotised sulphanilic acid in an alkaline medium of NaOH. The product of orangeazo dye formed proves to be intense, water–soluble and stable.
Materials and Methods

Apparatus

Shimadzu UV-Vis.Recording spectrophotometric had been used in measurement of absorbance using 1-cm silica cells.

Reagents

chemical and solvents are chose with high purity.

Working OMCl solution, 100 μg / ml. A 0.01g of OMCl is dissolved in distilled water and then volume is completed to 100 ml in a calibrated flask.

Diazotised sulphanic acid reagent solution, 30mM. A 0.5190 g of sulphanic acid is dissolved 75 ml of distilled water and the mixture is heated until the clear solution is obtained, then 1 ml of hydrochloric acid (conc.) is added, the mixture is left in ice bath at 0 - 5°C and 0.207g sodium nitrite is added and stirred vigorously and then the volume completed to 100 ml in a volumetric flask using cooled distilled water, and is stored in refrigerator. This solution is prepared freshly each day [17].

Alkaline solution of NaOH(1N), is prepared by This solution is prepared by appropriate dilution of the concentrated (Fluka) solution with distilled in a plastic container.

Nazodrin drops, (100μg / ml). Three containers of drug (each contains 10 ml of 0.05% OMCl) are mixed, then 20 ml of the above solution was diluted with distilled water to 100 ml in a volumetric flask to prepare a solution of 100 μg /ml OMCl.

Oxymet drops, (100μg/ml). Provided from the Pharaonia pharmaceuticals. Three containers of drug (each contains 15 ml of 0.025% OMCl) are mixed, then 40 ml of the above prepared solution was diluted to 100 ml by adding distilled water in a volumetric flask.

Procedure and calibration graph.

To a series of 25 ml volumetric flasks add 20 – 500 μg (0.8 – 20 ppm) of OMCl, 0.5 ml of diazotised sulphanic acid (30mM) and 1ml of 1N NaOH are then added and the volumes are diluted to the mark using distilled water as a slovent. After 10 minutes the A are read versus a reagent blank at 496 nm using 1-cm cell. The linearity is over the range 20 to 400 μg/25ml (0.8–16 ppm) (Fig.1). The molar absorptivity is found to be $2.30 \times 10^4 \text{l.mol}^{-1}\cdot\text{cm}^{-1}$

![Graph](attachment:image.png)

Fig.1. Calibration curve of OMCl determination

Results and Discussion

A 100μg of OMCl has been taken and final volumes are brought to 25 ml with distilled water in subsequent experiment
Absorption spectra.
When OMCl in aqueous solution is treated with diazotized sulphanilic acid reagent solution, an absorption peak is obtained showing an intense orange dye with maximum absorption at 496 nm. The reagent blank shows no absorption at this wavelength (Fig.2).

![Absorption spectrum of OMCl with diazotized sulphanilic acid at 496 nm](image)

Fig.2: Absorption spectrum of OMCl with diazotized sulphanilic acid at 496 nm
(A) the azo dye against blank, (B) blank against distilled water

Study of the optimum reaction conditions. All parameters effecting and related to orange azo dye have been optimized.

Effect of base. The preliminary experiments have shown that the azo dye develops only completely in using base solution. Different amounts of bases (strong and weak) have been used (Table 1).

| Alkaline solution (IN) | Variable | A / ml of various base used |
|------------------------|----------|----------------------------|
|                        |          | 0.1 | 0.3 | 0.5 | 0.7 | 1   | 1.2  | 1.5  |
| NaOH                   | A        | 0.283 | 0.271 | 0.259 | 0.247 | 0.263 | 0.199  | 0.161 |
|                        | *Δλ*, nm | 112 | 113 | 198 | 202 | 202 | 202 |
| KOH                    | A        | 0.119 | 0.127 | 0.137 | 0.162 | 0.206 | 0.166 | 0.163 |
|                        | Δλ*, nm  | 114 | 114 | 192 | 192 | 196 | 198 |
| Na₂CO₃                 | A        |       | No color contrast |
|                        | Δλ*, nm  |       | |
| NaHCO₃                 | A        |       | No color contrast |
|                        | Δλ*, nm  |       | |

\*Δλ = λ_{max} - λ_{max}B, where S = The azo dye and B = The Blank

The results in Table 1 indicated that 1ml of 1N sodium hydroxide is the more suitable amount which gives a high values of colour contrast.
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**Effect of diazotized sulphanilic acid reagent amount.**

Various volumes of the diazotized sulphanilic acid (30mM) are tested, the results indicate that using 0.5 ml of diazotized sulphanilic acid reagent solution gives maximum A of the complex at 496 nm and the volume is considered as an optimum value (Table 2).

**Table 2. The optimize amount of diazotised sulphanilic acid**

| Ml of diazotised sulphanilic acid reagent solution (30 mM) | Absorbance |
|----------------------------------------------------------|------------|
| 0.05                                                     | 0.144      |
| 0.1                                                      | 0.231      |
| 0.25                                                     | 0.242      |
| 0.5                                                      | 0.272      |
| 1.0                                                      | 0.266      |
| 1.5                                                      | 0.215      |
| 2.0                                                      | 0.122      |

**Effect of surfactant**

Three orders by using 1 ml of various types of surfactants have been studied. The effects of different surfactants on the colour intensity are showed that no useful effect and a loss in colour intensity are observed. Therefore, it has been recommended to eliminate the use of surfactants in the subsequent experiments (Table 3).

**Table 3. Effect of surfactants**

| Surfactant used | A* / order** of addition | I | II | III |
|-----------------|--------------------------|---|----|-----|
|                 |                          | A | Δλ | A   | Δλ  | A   | Δλ  |
| CTAB, 1×10^{-3}M | 0.075                    | 204| 0.080| 202 | 0.098| 204 |
| SDS, 1×10^{-3}M | 0.258                    | 198| 0.269| 200 | 0.262| 198 |
| Triton X-100, 1% | 0.181                    | 206| 0.176| 206 | 0.210| 208 |

* Absorbance without surfactant = 0.273
** 1. OMCi (O) + Surfactant (S) + Diazotised sulphanilic acid (R) + NaOH (OH)
   II. O + R + S + OH
   III. O + R + OH + S
***Δλ = λ_{max} S - λ_{max} B

**Stability of formed azo dye**

The color development showed that the colour started to form within about five minutes. The formation of azo dye being complete after 15 minutes and the absorbance of the coloured species remained constant for at least 25 minutes, this stability period is sufficient for several measurements (Table4).
Table 4. Effect of time and OMCl amount on A

| µg of OMCl present | Absorbance / minute standing time |
|-------------------|----------------------------------|
|                   | 5      | 10     | 15     | 20     | 25     | 30     | 40     | 50     | 60     |
| 50                | 0.129  | 0.187  | 0.211  | 0.211  | 0.207  | 0.203  | 0.197  | 0.178  | 0.168  |
| 100               | 0.249  | 0.277  | 0.307  | 0.309  | 0.311  | 0.310  | 0.299  | 0.276  | 0.255  |

Accuracy and precision

In order to check the accuracy and precision of the proposed method three various amounts of OMCl where taken and determined. The results illustrated in tables show that suggested method gave satisfactory.

Table 5. Accuracy and precision of the method

| Amount of OMCl taken, µg/25ml | Relative error, %* | Relative standard deviation, %* |
|-------------------------------|---------------------|---------------------------------|
| 40                            | +0.97               | ±1.57                           |
| 100                           | +0.21               | ±0.32                           |
| 200                           | -0.28               | ±0.27                           |

*Average of 5 determinations.

Nature of the dye.

Job’s method indicated that the azo dye has a composition of 1:1 OMCl to diazotized sulphanilic acid [R](Fig.3).

![Fig. 3: Job’s plot for OMCl – sulphanilic acid](image)
Hence the dye might be the following structure.

![Chemical structure of oxymetazoline hydrochloride](image)

**Fig. 5: The possible structure of the orange azo dye**

**Interference**

The excipients which frequently accompany pharmaceutical formulation are studied by adding three various amounts (100, 500 and 1000μg) to 100μg OMCl (Table 6).

**Table 6. Effect of foreign compounds for assay of OMCl**

| Foreign compound     | Recovery (%) of 100μg OMCl per μg foreign compound added in 25 ml |
|----------------------|---------------------------------------------------------------|
|                      | 100 | 500 | 1000 |
| Glucose              | 105.5 | 98.5 | 96.2 |
| Lactose              | 99.2 | 95.1 | 99.6 |
| Starch               | 98.8 | 102.9 | 101.1 |
| Gum Arabic (Acacia)  | 102.9 | 105.5 | 102.6 |

Table 6 with its results indicated that there is no interference of any excipients added to the determination of OMCl using the suggested procedure.

**Applications part**

The formulations of OMCl as nasal drops have been selected in our applications part. Good recoveries are obtained (Table 7).

**Table 7. Result of applications part.**

| Drug                              | μg OMCl present/25ml | μg OMCl measured/25ml | Recovery, % |
|-----------------------------------|----------------------|-----------------------|-------------|
| **Nazordin 0.05% S.D.I-Iraq**     | 50                   | 52.2                  | 104.5       |
|                                   | 100                  | 104.4                 | 104.4       |
|                                   | 200                  | 195.3                 | 97.6        |
| **Oxymet 0.025% Pharaonia(Egypt)**| 50                   | 49.5                  | 99.0        |
|                                   | 100                  | 105.2                 | 105.2       |
|                                   | 200                  | 204.7                 | 102.3       |

*Average of three determinations.
Evaluation of the proposed method

In order to prove that the suggested method can be applied to the determination of OMCl in formulations without interferences, a standard addition method is applied. The results in Fig.6 and Table 8 shows that there is no significant different between the amounts taken and experimental results.

![Graph](image)

**Fig. 6: Calibration standard addition graphs for the determination of OMCl in Nazordin [A] and Oxymet [B]**

| Drug | µg OMCl present/25ml | µg OMCl measured/25ml | Recovery, % |
|------|----------------------|-----------------------|-------------|
| **Nazordin 0.05% S.D.I-Iraq** | | | |
| 50 | 49.4 | 98.8 |
| 100 | 97.9 | 97.9 |
| **Oxymet 0.025% Pharaonia(Egypt)** | | | |
| 50 | 49.5 | 99.0 |
| 100 | 99.9 | 99.9 |

* Average of three determinations

**Variable Comparison**

The various analytical parameters for our method and the same parameters for other literature methods(14,16) have been calculated and illustrated in Table 9.
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| Table 9. Comparison of the methods |
|-----------------------------------|
| **Anal. Parameters** | **Present method** | **Lit. method**<sup>(16)</sup> | **Lit. method**<sup>(14)</sup> |
| pH | 11.73 | … | … |
| Temperature (C°) | At room temperature | 70 | 70 |
| λ<sub>max</sub> (nm) | 496 | 480 | 510 |
| Medium of reaction | Aqueous | Aqueous | Aqueous |
| Type of reaction | Diazot coupling | Oxidative coupling | Redox reactions |
| Reagent | Diazotised sulphanilic acid | 4-Amino-antipyrine | 1,10-phenanthroline |
| Beer’s law range (ppm) | 0.8-16 | 1-20 | 0.1-7 |
| Molar absorbptivity (l.mol<sup>-1</sup>.cm<sup>-1</sup>) | 2.03 × 10<sup>4</sup> | 5.34×10<sup>4</sup> | 5.74×10<sup>4</sup> |
| Nature of the dye | 1:1 | 1:2 | 1:1 |
| Application of the method | Nazordin 0.05% | Nazordin 0.05% | Nazordin 0.05% |
| | Oxymet 0.025% | Oxymet 0.025% | Oxymet 0.025% |

## Conclusion

The proposed method is simple, sensitive and there is no previous separation or temperature controlled. The method has been successfully applied to the determination of OMCl in various pharmaceutical preparations.

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