Atomic absorption spectrometric determination of Fusidic Acid in bulk powder and in pharmaceutical dosage form

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ABSTRACT
Atomic absorption spectrometric method is based on precipitation of the ion associates formed from the reaction of fusidic acid with silver nitrate (procedure A), copper acetate (procedure B) or ferric chloride (procedure C) standard solutions. The formation and solubility of the solid complexes at the optimum conditions of pH and ionic strength values have been studied. The method depends on direct determination of the ions in the precipitate or indirect determination of the ions in the filtrate by atomic absorption spectroscopy. The optimum conditions for precipitation were carefully studied. Rectilinear calibration graphs were obtained in the range of 20-100 ng ml\(^{-1}\) for the investigated drug. The molar ratios of the formed chelats were determined by Job's method and their association constants were also calculated. The developed method (procedures I, II and III) were applied successfully for the determination of the studied drug in its pharmaceutical dosage form with a good precision and accuracy compared to the reported method as revealed by t-and F-tests.

Keywords: Fusidic acid; Atomic absorption spectroscopy; Bulk powder; Pharmaceutical analysis.

INTRODUCTION
Fusidic acid is antibiotic derived from Fusidium coccineum, exerts powerful antibacterial activity against a number of gram-positive organisms. Staphylococci, including the strains resistant to penicillin or other antibiotics, are particularly susceptible to fusidic acid [1].

Few papers have been reported for determination of fusidic acid include, voltametry [2, 3], liquid chromatography [4], high performance liquid chromatography [5-8] and spectrophotometry [9-12].
Although atomic absorption spectrometry is a rapid method and has very low detection limits which cannot be reached by most of other methods, it has not been applied yet to the determination of fusidic acid. The present work includes new direct and indirect methods for determination of fusidic acid. The present work represents the utilization of silver nitrate, copper acetate and ferric chloride as reagents for the determination of the studied drug by direct and indirect atomic absorption spectrometric measurements. The methods proved to be very sensitive and accurate for the determination of fusidic acid in bulk powder and in pharmaceutical dosage forms.

In continuation, I wish here to develop simple and rapid selective atomic absorption spectrometric procedures for the determination of fusidic acid in bulk power and in pharmaceutical dosage forms.

**EXPERIMENTAL SECTION**

2. 1. **Apparatus**
- Spectronic™ Genesys™, UV/VIS spectrophotometer connected to an IBM computer loaded with the Winspec™ application software.
- A Shimadzu atomic absorption flame spectrophotometer model AA.640-13. For AAS, silver, copper and iron were measured at wavelengths 328.1, 327.7 and 240.7 nm, respectively, slit width 0.2 nm, relative noise 1.0, detection limit 0.01 µg/ml, lamp current 7 mA and integration time 3 s. The flame used was the acetylene-air mixture.
- The pH values of solutions were measured using an Orion Research Model 601A digital pH-meter.

All calculations were carried out on IBM computer using Microsoft excel 2002 for windows ME. SMAC program [13] was used for all statistical methods.

2. 2. **Chemicals and reagents**
All solvents and reagents were of analytical reagent grade, double distilled water was used throughout. Fusidic was generously supplied by their respective manufacturers (LEO Pharmaceutical Products, DENMARK). 0.025 M silver nitrate (0.524% W/V solution), 0.01 M copper acetate (0.2% W/V solution) and 0.01 M ferric chloride (0.18% W/V solution) were Aldrich products.

2.3. **Pharmaceutical preparation**
Fucicort® cream (LEO Pharmaceutical Products, DENMARK) labeled to contain 20 mg fusidic acid and 1 mg betamethasone per each game of cream.

2. 4. **Standard preparations**
Stock solutions containing 10 µg ml⁻¹ fusidic acid was prepared in distilled water. Working standard solutions containing 20-100 ng ml⁻¹, were prepared by suitable dilution of the stock solutions with distilled water.

2.5. **Procedures**
2.5.1. **Atomic absorption spectrometric method utilizing silver (Procedure A)**
To aliquots of fusidic acid stock solution (equivalent to 20-100 ng ml⁻¹), two ml of 0.025 M silver nitrate solution was added. Solutions were protected from light, shaken and filtered (whatman No.44). The precipitates were washed with redistilled deionized water until free of silver (I).
2.5.1.1. Direct method
The precipitates obtained above were dissolved in a minimum amount of dilute ammonia solution and completed to 25 ml with redistilled deionized water. Two ml of the resulting solutions was diluted to 25 ml with redistilled deionized water.

2.5.1.2. Indirect method
The filtrates and washings were collected in a 100 ml volumetric flask and completed to volume with redistilled deionized water. Ten ml of the resulting solution was diluted to 100 ml with redistilled deionized water.

A blank (omitting addition of drug) was prepared and the absorbance was measured at the flaming conditions; wavelength 328.1 nm, lamp current 7 mA, slit width 3.8 A, air flow rate 10L/min and acetylene flow rate 2.6 L/min. Silver (I) concentrations were calculated from a calibration curve.

2.5.2. Atomic absorption spectrometric method utilizing copper (Procedure B)
To aliquots of fusidic acid stock solution (equivalent to 20-100 ng ml\(^{-1}\)), two ml of copper acetate solution was added. Solutions were well shaken, filtered (whatman No.44), and the precipitates were washed with redistilled deionized water until free of copper (II).

2.5.2.1. Direct method
Precipitates were dissolved in a minimum amount of dilute ammonia solution and completed to 100 ml with redistilled deionized water. Five ml of the resulting solution was transferred into a 50 ml volumetric flask and completed to volume with redistilled deionized water.

2.5.2.2. Indirect method
The filtrates and washings were collected in a 100 ml volumetric flask and completed to volume with redistilled deionized water. Five ml of the resulting solution was diluted to 100 ml with redistilled deionized water.

A blank (omitting addition of drug) was prepared and the absorbance was measured at the flaming conditions; wavelength 327.7 nm, lamp current 7 mA, slit width 3.8 A, air flow rate 10L/min and acetylene flow rate 2.3 L/min. Copper (II) concentrations were calculated from a calibration curve.

2.5.3. Atomic absorption spectrometric method utilizing iron (Procedure C)
To aliquots of fusidic acid stock solution (equivalent to 20-100 ng ml\(^{-1}\)), two ml of ferric chloride solution was added, shaken well and filtered (whatman No.44). The precipitates were washed with redistilled deionized water until free of iron (III).

2.5.3.1. Direct method
The precipitates were dissolved in a minimum amount of dilute ammonia solution and completed to 25 ml with redistilled deionized water. Two ml of the resulting solutions was diluted to 50 ml with redistilled deionized water.

2.5.3.2. Indirect method
The filtrates and washings were collected in a 100 ml volumetric flask and completed to volume with redistilled deionized water. Five ml of the resulting solution was diluted to 100 ml with redistilled deionized water.
A blank (omitting addition of drug) was prepared and the absorbance was measured at the flaming conditions; wavelength 240.7 nm, lamp current 7 mA, slit width 3.8 Å, air flow rate 10L/min and acetylene flow rate 2.5 L/min. Iron (III) concentrations were calculated from a calibration curve.

2.5.4. For pharmaceutical preparations
Evacuate the contents of five tubes and extract the fusidic acid contents with ethanol and complete as under 2.5.

2.5.5. For stoichiometric relationship
Drug and metal solutions of equimolar concentrations ($1\times10^{-4} M$) were prepared. Aliquots of each solution were added in different ratios to a series of 10 ml calibrated flasks, so that the total volume of both is 5 ml. The pH is adjusted using 1 ml buffer solution [17] and then the volume is completed with the appropriated solvent. The relative absorption intensity of each formed chelate is measured at its respective maxima [18].

RESULTS AND DISCUSSION

Slightly alkaline (pH 7.8-8.3) alcoholic solutions of fusidic acid gave white coagulated precipitates with silver nitrate (procedure A), green bluish precipitates with copper acetate (procedure B) and reddish brown precipitates with ferric chloride (procedure C). These precipitates form the basis of the micro-quantitative determinations of sildenafil citrate. Ag (I), Cu (II) or Fe (III) contents can be determined either directly in the precipitate or indirectly in the filtrate by atomic absorption spectrometry.

3. 1. Optimization of the reaction conditions
3.1.1. Type and amount of alcohol
Addition of the recommended amount of ethyl alcohol is to enhance the solubilization of the drug and coagulation of the precipitates. Larger volumes of alcohol must be avoided to prevent solubilization of the formed precipitates.

3.1.2. Effect of pH
In order to study the effect of pH on precipitation, buffer solutions covering the acid to alkaline range were tried. Acid media have a solubilizing effect on the precipitate leading to lower results for the direct technique and higher ones for the indirect technique while higher alkali media precipitate the metal as its oxide or hydroxide leading to higher results for the direct technique. The optimum pH was found to be slightly alkaline (pH = 7.8 – 8.3).

3.1.3. Metal concentration
Considering metal ion concentration effect on precipitation, 2 ml of the precipitating solutions was found to be sufficient for complete precipitation.

3.1.4. Temperature
Regarding the temperature effect on precipitation, room temperature was found to be the most efficient. Higher temperature show solubilizing effect on the precipitate producing lower results for the direct technique and higher ones for the indirect technique.
3.1.5. Composition of the formed complex

Job's method of continuous variation [18] was used to study the molar ratios of the formed chelates. The method revealed 1:1, 2:1 and 3:1 fusidic acid to silver (I), copper (II) and iron (III), respectively.

The stability constants of the formed chelates were calculated using the following equations:

\[
\beta = \frac{A/A_{ex} C_X}{(C_M - A/A_{ex} C_X) (C_L - nA/A_{ex} C_X)^n}
\]

Where \( \beta \) is the stability constant of the formed chelate, \( M \) indicates metal, \( L \) indicates ligand, \( n = X/(1-X) \) where \( X \) is the mole fraction of the ligand at the maximum of the continuous variation curve. \( A/A_{ex} \) is the ratio of the observed absorbance to that indicated by the tangent for the same wavelength. \( C_M \) and \( C_L \) are the concentrations of the metal and the ligand, respectively, \( C_X = C_L/n = C_M \) [19].

The calculated stability constants for the formed chelates (Table 1) are ranging from \( 111.1482 \times 10^{-7} \) to \( 179.2123 \times 10^{-7} \) indicating good stability of the formed chelates.

<table>
<thead>
<tr>
<th></th>
<th>Ass. Const. x 10^{-7}</th>
<th>Log (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver</td>
<td>179.2123</td>
<td>9.0598</td>
</tr>
<tr>
<td>Copper</td>
<td>111.1482</td>
<td>9.1868</td>
</tr>
<tr>
<td>Iron</td>
<td>125.6947</td>
<td>9.0198</td>
</tr>
</tbody>
</table>

Average of three determinations

3.1.6. Stoichiometric relationship

Job's method of continuous variation [18] indicated molar ratios of 1:1, 2:1 and 3:1 fusidic acid to silver (I), copper (II) and iron (III), respectively (Figure 1).

![Figure 1: Continuous variation plot of fusidic acid with silver (−), copper (−−) and iron (−−−) salts.](image-url)
3.2. Quantification and validation of assay procedures

3.2.1. Accuracy
To study the accuracy of the proposed methods, synthetic mixtures containing various amounts of drug were prepared and analyzed by the proposed atomic absorption spectrometric (A, B and C) Also, the C.V. % was calculated from the results of recovery experiments (Table 2).

<table>
<thead>
<tr>
<th>Conc. range</th>
<th>a</th>
<th>b</th>
<th>r</th>
<th>r²</th>
<th>LOD</th>
<th>LOQ</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic absorption spectrometric method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedure A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct 20-100 ng ml⁻¹</td>
<td>0.143</td>
<td>0.213</td>
<td>0.9994</td>
<td>0.9992</td>
<td>1.211</td>
<td>3.392</td>
<td>0.42</td>
</tr>
<tr>
<td>Indirect 20-100 ng ml⁻¹</td>
<td>-1.032</td>
<td>0.146</td>
<td>0.9996</td>
<td>0.9992</td>
<td>1.219</td>
<td>4.150</td>
<td>0.78</td>
</tr>
<tr>
<td>Procedure B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct 20-100 ng ml⁻¹</td>
<td>0.128</td>
<td>0.121</td>
<td>0.9997</td>
<td>0.9994</td>
<td>1.431</td>
<td>4.492</td>
<td>0.32</td>
</tr>
<tr>
<td>Indirect 20-100 ng ml⁻¹</td>
<td>0.521</td>
<td>0.432</td>
<td>0.9996</td>
<td>0.9992</td>
<td>1.231</td>
<td>3.298</td>
<td>0.98</td>
</tr>
<tr>
<td>Procedure C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct 20-100 ng ml⁻¹</td>
<td>0.298</td>
<td>0.126</td>
<td>0.9995</td>
<td>0.9990</td>
<td>1.389</td>
<td>3.298</td>
<td>0.11</td>
</tr>
<tr>
<td>Indirect 20-100 ng ml⁻¹</td>
<td>0.8765</td>
<td>0.911</td>
<td>0.9998</td>
<td>0.9996</td>
<td>1.231</td>
<td>2.988</td>
<td>0.65</td>
</tr>
</tbody>
</table>

a: intercept; b: slope; r: correlation coefficient; r²: coefficient of determination; LOD: limit of detection; LOQ: limit of quantitation.

The accuracy of the proposed procedures were established by comparing the results of analysis of the proposed procedures with the reported method [12], the suggested methods are equally precise and accurate to the reported method (Table 3).

3.2.2. Precision
The precision of the methods were tested by triplicate analysis of different concentrations. For comparison, the reported method [12] was applied for the determination of the intact drug. Statistical analysis of the results obtained (Table 3) indicated that the proposed procedures were as accurate and precise as the reported method.

<table>
<thead>
<tr>
<th></th>
<th>X</th>
<th>±SD</th>
<th>N</th>
<th>V</th>
<th>t</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported method [12]</td>
<td>99.64</td>
<td>0.91</td>
<td>6</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedure A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>100.5</td>
<td>1.75</td>
<td>6</td>
<td>1.81</td>
<td>0.81</td>
<td>2.12</td>
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<tr>
<td>Indirect</td>
<td>100.3</td>
<td>1.22</td>
<td>6</td>
<td>1.31</td>
<td>0.77</td>
<td>2.77</td>
</tr>
<tr>
<td>Procedure B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>99.71</td>
<td>0.24</td>
<td>6</td>
<td>1.15</td>
<td>1.81</td>
<td></td>
</tr>
<tr>
<td>Indirect</td>
<td>100.1</td>
<td>1.57</td>
<td>6</td>
<td>1.17</td>
<td>0.62</td>
<td>2.92</td>
</tr>
<tr>
<td>Procedure C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>99.4</td>
<td>0.65</td>
<td>6</td>
<td>0.41</td>
<td>0.62</td>
<td>1.74</td>
</tr>
<tr>
<td>Indirect</td>
<td>100.7</td>
<td>1.15</td>
<td>6</td>
<td>1.72</td>
<td>2.21</td>
<td>1.54</td>
</tr>
</tbody>
</table>

The t- and F- values refer to comparison of the proposed method with the official or reported methods. Theoretical values at 95% confidence limit t = 2.23 and F = 5.79.

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3.2.3. Detection limit
By applying the proposed analytical procedures, the limit of detection was detected both practically and by mathematical method. It was calculated according to the recommendation for LOD for spectrochemical methods which was published in 1987 by international union of pure and applied chemistry IUPAC [20]. The practically determined LOD did not show significant different from that of calculated one (Table 2).

3.2.4. Quantitation limit
It can be calculated using SMAC program [16], but it must be also determined practically by analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision (Table 2).

The practically determined LOQ was showed significant different from that of calculated one as it is little higher than that of calculated ones.

3.2.5. Linearity range
A linear correlation was found between absorbance and concentration of fusidic acid in the range 20-100 ng ml\(^{-1}\) for procedures (A, B and C). The linearity range can be determined by making a plot of absorbance as a function of concentrations for both drug and reagents (Table 2).

3.2.6. Ruggedness and robustness
The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of conditions while robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters.

Both ruggedness and robustness were determined by assessment of the degree of reproducibility of the regression analysis obtained by determination of the same standard samples in different days as the regression equation was determined at each \(\lambda_{\text{max}}\) along different four weeks (Table 4), there is no significant difference between the regression equations that determined along the four weeks, this provide that these methods used for determination of the studied drug are reproducible and not affected by variations in the day of analysis.

<table>
<thead>
<tr>
<th>Method</th>
<th>Conc. Range</th>
<th>Intercept</th>
<th>Zero Time</th>
<th>1 Week</th>
<th>2 Week</th>
<th>3 Week</th>
<th>Mean</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proc. A</td>
<td>20-100 ng ml(^{-1})</td>
<td>0.0011</td>
<td>0.0021</td>
<td>0.0022</td>
<td>0.0031</td>
<td>0.0045</td>
<td>0.0014</td>
<td></td>
</tr>
<tr>
<td>Proc. B</td>
<td>20-100 ng ml(^{-1})</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0009</td>
<td>0.0023</td>
<td>0.0032</td>
<td>0.0055</td>
<td></td>
</tr>
<tr>
<td>Proc. C</td>
<td>20-100 ng ml(^{-1})</td>
<td>0.0005</td>
<td>0.0015</td>
<td>-0.0011</td>
<td>0.0086</td>
<td>0.0062</td>
<td>0.0029</td>
<td></td>
</tr>
</tbody>
</table>

3.3. Analysis of pharmaceutical formulations
The proposed atomic absorption spectrometric procedures were applied to the determination of fusidic acid in Fucicort® Cream (Table 5). The results were compared statistically with those obtained by applying the reported methods [12].
In the t and F tests, no significance difference was found between the calculated and theoretical values (95% confidence) of the proposed and reported methods. This indicates similar precision and accuracy. Data of Table 5 suggested that the present procedures could be applied to the assay of this drug in its single dosage form without interference. Frequently encountered common ingredients of formulations were found not to interfere.

The obtained high-intensity absorption bands and the very low reagent background make this procedure suitable for the routine quality control analysis of the investigated sildenafil citrate drug with minimum interference.

### Table 5: Determination of fusidic acid in Fucicort® Cream

<table>
<thead>
<tr>
<th></th>
<th>X</th>
<th>±SD</th>
<th>N</th>
<th>V</th>
<th>t</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported method [12]</td>
<td>99.64</td>
<td>0.91</td>
<td>6</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedure A Direct</td>
<td>99.6</td>
<td>1.28</td>
<td>6</td>
<td>2.10</td>
<td>0.23</td>
<td>1.23</td>
</tr>
<tr>
<td>Indirect</td>
<td>100.4</td>
<td>1.22</td>
<td>6</td>
<td>1.41</td>
<td>0.78</td>
<td>1.87</td>
</tr>
<tr>
<td>Procedure B Direct</td>
<td>99.1</td>
<td>1.98</td>
<td>6</td>
<td>1.91</td>
<td>0.92</td>
<td>1.12</td>
</tr>
<tr>
<td>Indirect</td>
<td>100.3</td>
<td>0.94</td>
<td>6</td>
<td>1.67</td>
<td>0.39</td>
<td>1.67</td>
</tr>
<tr>
<td>Procedure C Direct</td>
<td>99.00</td>
<td>1.13</td>
<td>6</td>
<td>0.93</td>
<td>0.59</td>
<td>1.89</td>
</tr>
<tr>
<td>Indirect</td>
<td>100.2</td>
<td>1.45</td>
<td>6</td>
<td>1.34</td>
<td>0.89</td>
<td>1.98</td>
</tr>
</tbody>
</table>

The t- and F-values refer to comparison of the proposed method with the official or reported methods. Theoretical values at 95% confidence limit t = 2.23 and F=5.79.

**CONCLUSION**

Simple, rapid, accurate sensitive and selective atomic absorption spectrometric methods were developed for the analysis of fusidic acid via its reaction with the suggested reagents. Moreover, all the applied methods are much more sensitive than reference method. The proposed procedures could be applied to quality-control analysis of the investigated drug. The procedures could also be automated, the ongoing work is directed to develop and validate this system.

**REFERENCES**


