REDOX METHODS VALIDATION OF PARACETAMOL AND ASCORBIC ACID IN PHARMACEUTICAL PREPARATIONS

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Abstract: The contents of active substances were determined in a preparation TP-4 (tablets) containing paracetamol, ascorbic acid, caffeine and phenylephrine hydrochloride. For the determination of paracetamol and ascorbic acid a non-specific (cerometric and titration of 2,6-dichloroindophenol) method based on a redox reaction was used. Validation of the methods, performed on model mixtures, proved those methods to be accurate, precise, reproducible and linear within the range from 50% to 150% of the amount declared in the preparation. The content of paracetamol and ascorbic acid in TP-4, Thomaprynas®, Panadol Extra, Ring N, Polopiryna C®, Efferalgan Vitamine C and Vitaminum C 0.2 satisfies the FP V demands (±10% of the declared amount).

Keywords: paracetamol, ascorbic acid, validation, cerometric method, titration of 2,6-dichloroindophenol.

Paracetamol, ascorbic acid, caffeine and phenylephrine hydrochloride are frequently used components of antiinflammatory, anti-inflammatory and antipyretic drugs. The preparations combining these components offer the advantage of parallel activity of all four components and are in great demand. However, similar physical and chemical properties make identification and exact determination of these four components, in the presence of impurities difficult.

The quantitative analysis of pharmaceutical preparations is based on physical and chemical properties of the active substance and on the reactivity of groups in the molecule analysed.

The amide group present in the molecule allows paracetamol to be determined alkalimetric ally in an unhydrous medium (1,2), whereas the sensitivity of the phenol group to oxidising agents has been used in the cerometric (1–3) and polarographic (1,2) methods of determination. The possibility of release of an aromatic amide group as a result of acid hydrolysis of aniline derivatives is used in the method of titrimetric method of paracetamol determination (1,2) and colorimetric analysis after condensation with aldehydes (2). Reaction of nitration or oxidation with sodium chlorate (2) can also be used in colorimetric determination (2).

The endiol group present in the molecule allows ascorbic acid to be determined alkalimetric (3) and redox methods. The sensitivity to oxidising agents (pH = 1.2, $\pi^+ = +0.105$ V; pH = 5.1, $\pi^+ = +0.017$ V; pH = 5.1, $\pi^+ = +0.232$ V; pH = 9.2, $\pi^+ = +0.305$ V) (1,4) has been used in the iodometric (1.3–5), cerometric (1), polarographic (4), titration of 2,6-dichloroindophenol (1,4,5) and in the colorimetric method of determination (6,7).

In this paper we propose redox methods (cerometric and titration of 2,6-dichloroindophenol) for the determination of paracetamol and ascorbic acid in a pharmaceutical preparation containing: paracetamol (0.450 g), ascorbic acid (0.030 g), caffeine (0.025 g), phenylephrine hydrochloride (0.005 g) and in other pharmaceutical preparations.

EXPERIMENTAL

Materials and reagents

Ascorbic acid and paracetamol were purchased from Sigma–Aldrich, Germany; Cefalgin, s. 100996, Pabianickie Zakłady Farmaceutyczne Polfa, Poland; Efferalgan Vitamine C, s. 6 230, UPSA, France; Panadol Extra, s. 3PF870, SmithKline Beecham, England; Polopiryna C®, s. 81197, Polfarmma S.A., Stargard Gdaski, Poland; Ring N, s. 51052000, Heirich Mack Nach, Germany; Thoma-pyrin®, s. 701633, Boehringer Ingelheim International GmbH, Germany; Vitaminum C 0.2, s. 010197, Krakowskie Zakłady Farmaceutyczne Polfa S.A., Poland.

Placebo: potato starch, purified talc, magnesium stearate, silicon dioxide colloidal mowiol 8–88.

All the chemicals used were analytical reagent grade.
RESULTS

1. The cerometric method validation

1.1. Solutions

Cerium (IV) sulphate: weigh accurately about 21 g cerium (IV) sulphate, add 15 ml of water and 14 ml of sulphuric acid (1.762 kg/l), mix, add 200 ml of water and heat to dissolution. Transfer the solution to a 500 ml volumetric flask, dilute with water to 500.0 ml and filter.

Ortho-phananthroline: weigh accurately about 0.7 g ferrum (II) sulphate and 1.6 g o-phananthroline hydrochloride, dissolve in 60 ml of water and dilute with water to 100.0 ml.

Estimation of the concentration cerium (IV) sulphate solution: weigh accurately about 80 mg potassium iodide, add 25 ml of water, 5 ml of sulphuric acid (698 g/l), 0.15 ml of o-phananthroline solution and 10 ml of acetone, mix and titrate with cerium (IV) sulphate solutions to change of colour from red to blue.

1.2. Specificity

Caffeine, acetylsalicylic acid, ascorbic acid, paracetamol, phenylephrine hydrochloride, propyphenasone or placebo were weighed accurately to about 30 mg, then dissolved in 2 ml of ethanol (760 g/l) and added to 30 ml of hydrochloric acid (105 g/l), 0.1 ml of o-phananthroline solution and titrated with cerium (IV) sulphate solution. The volumes of cerium (IV) sulphate solution were: 0.04 ml for 32.2 mg of caffeine, 0.11 ml for 35.8 mg of acetylsalicylic acid, 4.83 ml for 35.2 mg of ascorbic acid, 15.63 ml for 32.1 mg of paracetamol, 9.00 ml for 33.0 mg of phenylephrine hydrochloride, 9.48 ml for 33.4 mg of propyphenasone, 0.05 ml for 31.0 mg of placebo.

The cerometric method determinations of paracetamol in mixture with ascorbic acid, acetylsalicylic acid and propyphenasone is non-selective.

1.3. Accuracy, precision and reproduction

The accuracy, precision and reproduction of the method were assessed on the basis of determinations of paracetamol in three model mixtures whose compositions are given in Table 1. Series of 6 or 9 determinations were made for each model mixture, according to the procedure described in section 1.2. The content of paracetamol (in g) was calculated by the cerometric method from the equation:

\[
x = \frac{V \cdot c \cdot 2.52}{0.1 \cdot 1000} \cdot \frac{M}{m}
\]

where:
- \( V \) – the volume of cerium (IV) sulphate solution, ml
- \( c \) – the concentration of cerium (IV) sulphate solution, mol/l
- \( M \) – the mean weight of a tablet or the mass of the model mixture, g
- \( m \) – the mass of weighed portion, g

<table>
<thead>
<tr>
<th>Model mixture</th>
<th>N</th>
<th>Determination and accuracy</th>
<th>Precision</th>
<th>Reproduction</th>
</tr>
</thead>
</table>
| Paracetamol 0.3149 g, Placebo 0.3930 g | 6  | 0.3170 ± 0.0059 g 100.7 ± 1.8 % | \( \sigma = 0.00560 \)  
\( \sigma_t = 0.00229 \)  
\( CV = 1.76\% \) | F = 1.64  
\( F_{10}(n_1-1,n_2-1) = 5.05 \)  
\( t = 0.299 \)  
\( t_{0.05}(n+n_2-2) = 2.228 \) |
| Paracetamol 0.4126 g, Placebo 0.2860 g | 9  | 0.4153 ± 0.0062 g 100.7 ± 1.5% | \( \sigma = 0.00807 \)  
\( \sigma_t = 0.00269 \)  
\( CV = 1.94\% \) |  |
| Paracetamol 0.5225 g, Placebo 0.1993 g | 6  | 0.5231 ± 0.0054 g 100.1 ± 1.1% | \( \sigma = 0.00516 \)  
\( \sigma_t = 0.00211 \)  
\( CV = 0.98\% \) |  |
|                     | 6  | 0.5239 ± 0.0042 g 100.3 ± 0.8% | \( \sigma = 0.00403 \)  
\( \sigma_t = 0.00165 \)  
\( CV = 0.76\% \) |  |
Table 2. Validation parameters of the ascorbic acid determination by method of titration with 2,6-dichloroindophenol

<table>
<thead>
<tr>
<th>Model mixture</th>
<th>N</th>
<th>Determination and accuracy</th>
<th>Precision</th>
<th>Reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid 0.0288 g Placebo 0.6110 g</td>
<td>9</td>
<td>0.0288 ± 0.0002 g 100.0 ± 0.7 %</td>
<td>σ = 0.000320  σn = 0.000107  CV = 1.11%</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid 0.0423 g Placebo 0.5632 g</td>
<td>6</td>
<td>0.0421 ± 0.0003 g 99.5 ± 0.7 %</td>
<td>σ = 0.000288  σn = 0.000118  CV = 0.68%</td>
<td>F = 1.66  F0.05(ν1=1,ν2=1) = 5.05  t = 0.400  t0.05(ν1+ν2=2) = 2.228</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.0421 ± 0.0004 g 99.5 ± 0.9 %</td>
<td>σ = 0.000387  σn = 0.000158  CV = 0.92%</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid 0.5568 g Placebo 1.2424 g</td>
<td>9</td>
<td>0.5566 ± 0.0036 g 100.0 ± 0.6 %</td>
<td>σ = 0.00472  σn = 0.00157  CV = 0.84%</td>
<td></td>
</tr>
</tbody>
</table>

The mean values of the accuracy, precision and reproduction (F. Snedecor and Student’s test) are given in Table 1.

1.4. Linearity

In order to check the linear relationship between the volume of cerium (IV) sulphate solution and the content of paracetamol in the weighed portions, five model mixtures were prepared of concentrations varying from 40.6% to 117.0% of paracetamol, relative to its content declared in the preparation TP-4. For each model mixture, three determinations were made according to the procedure given in section 1.2. The results were used for the calculation of the parameters of regression and correlation of the relationships: \( V = f(x) \), where \( x \) – the actual content of paracetamol in the weighed portion \( (a = 476.4 ± 23.3, b = -0.233 ± 0.519, r = 0.9951, σ_0 = 13.1, σ_n = 0.293, n = 15) \), and \( Y = f(x) \), \( Y \) – the content of paracetamol in the weighed portion (Figure 1).

Assuming the equation \( y = ax + b \), the regression coefficients \( a \) and \( b \) were determined and their significance assessed. The values of \( t = b/σ_b \) were found to be lower than the critical values \( t_{0.05}(f) \), which means that the value of \( b \) is not significant, so the direct cerometric methods of paracetamol determination is not charged with a systematic error and the relation \( V = f(x) \) can be described by the equation \( y = ax (a = 466.5 ± 27.2; r = 0.9951, n = 15) \). The direction coefficients of the lines \( Y = f(x) \) tend to 1 \( (a = 1.03 ± 0.05; r = 0.9950, n = 15) \), which confirms the accuracy of the method.

Figure 1. Plots of the content observed vs. actual content of paracetamol in the weighed portion

2. The titration of 2,6-dichloroindophenol

2.1. Solutions

2,6-Dichloroindophenol: weigh accurately about 0.25 g sodium salts of 2,6-dichloroindophenol, dissolve in 750 ml of warm sodium hydrogen carbonate solution (0.28 g/l), cool and dilute with water to 1000.0 ml.

Estimation of the concentration 2,6-dichloroindophenol solution: weigh accurately about 50 mg ascorbic acid, dissolve in a 60 ml of oxalic acid solution (20 g/l) and dilute to 100.0 ml. To 4.0 ml solutions, add 10 ml of sulfuric acid (177.5 g/l) and
titrate with 2,6–dichloroindophenol solutions to pink colour (5 s).

2.2. Specificity
Caffeine, acetylsalicylic acid, ascorbic acid, paracetamol, phenylephrine hydrochloride or placebo were weighed accurately to about 30 mg, dissolved in 8 ml of oxalic acid solution (20 g/l), added to 20 ml of sulphuric acid (177.5 g/l) and titrated with 2,6–dichloroindophenol solution. The volumes of 2,6–dichloroindophenol solution were:

0.12 ml for 31.2 mg of caffeine
0.11 ml for 34.1 mg of acetylsalicylic acid
26.21 ml for 2.95 mg of ascorbic acid
0.26 ml for 30.4 mg of paracetamol
0.19 ml for 29.4 mg of phenylephrine hydrochloride
0.21 ml for 34.1 mg of placebo.

The method of the determination of ascorbic acid in mixture with acetylsalicylic acid, caffeine, paracetamol, phenylephrine hydrochloride and placebo is selective.

2.3. Accuracy, precision and reproduction
The accuracy, precision and reproduction of the method was assessed on the basis of the determinations of ascorbic acid in three model mixtures whose composition are given in Table 2. Series of 6 or 9 determinations were made for each model mixture, according to the procedure:
a) for the ascorbic acid determinations 30 mg in tablets:
weigh accurately to about 40 mg model mixtures, dissolve in 8 ml of oxalic acid solution (20 g/l), add 20 ml of sulphuric acid (177.5 g/l) and titrate with 2,6–dichloroindophenol solutions.
b) for the ascorbic acid determinations 0.2 g in tablets:
weigh accurately to about 75 mg, dissolve in a 100 ml volumetric flask of oxalic acid solution (20 g/l). To 8.0 ml solutions, add 20 ml of sulfuric acid (177.5 g/l) and titrate with 2,6–dichloroindophenol solutions.

The content of ascorbic acid (in mg) were calculated by the titration method from the equation:

\[ x = 0.1086 \cdot V \cdot \frac{M}{m} \]

where:
V – the volume of 2,6–dichloroindophenol solution, ml
0.1086 – the quantity of ascorbic acid equivalent to 1.0 ml of 2,6–dichloroindophenol solution, mg

M – the mean weight of a tablet or the mass of the model mixture, g
m – the mass of weighed portion, g

The mean values of the accuracy, precision and reproduction (F. Snedecor and Student’s t-test) are given in Table 2.

2.4. Linearity
In order to check the linear relationship between the volume of 2,6–dichloroindophenol solution and the content of ascorbic acid in the weighed portions, five model mixtures were prepared of concentrations varying from 51.4% to 140.0% of ascorbic acid relative to its content declared in the preparation TP–4. For each model mixture, three determinations were made according to the procedure given in section 2.3.a. The results were used for the calculation of the parameters of regression and correlation of the relationship: \( V = f(x) \), where \( x \) – the actual content of ascorbic acid in the weighed portion (\( a = 9252.1 \pm 348.5, b = -0.0828 \pm 0.7170, r = 0.9971, \sigma_x = 196.7, \sigma_y = 0.450, n = 15 \)), and \( Y = f(x) \), \( Y \) – the content of ascorbic acid in the weighed portion (Figure 2).

Assuming the equation \( y = ax + b \), the regression coefficients \( a \) and \( b \) were determined and their significance assessed. The values of \( t = b/a \sigma \) were found to be lower than the critical values \( t_{0.05}(f) \), which means that the value of \( b \) is not significant, so the direct titration methods of ascorbic acid determination is not charged with a systematic error and the relation \( V = f(x) \) can be described by the equation \( y = ax \) (\( a = 9214.0 \pm 406.5, r = 0.9971, n = 15 \)) The direction coefficients of the lines \( Y = f(x) \) tend to 1 (\( a = 1.00 \))
± 0.03, \( r = 0.9971, n = 15 \), which confirms the accuracy of the method.

3. The determination of paracetamol and ascorbic acid in pharmaceutical preparations

Determinations of the contents of paracetamol and ascorbic acid in pharmaceutical preparations: TP–4, Thomapyrin\(^\text{®}\), Panadol Extra, Ring N, Polopiryna C\(^\text{®}\), Efferalgan Vitamine C and Vitaminum C 0.2, were made following the procedure described in section 1.2 and 2.2. Statistical analysis of the results is given in Table 3.

DISCUSSION

For the determination of paracetamol a non–specific cerometric method based on a redox reaction was used. Non–specificity of the method followed from the presence of ascorbic acid, showing strong reducing properties, and phenylephrine hydrochloride whose sensitivity to oxidisers was related to its phenol group. Validation of the method performed for model mixtures of paracetamol and placebo proved that for single–component preparations the cerometric method of paracetamol determination was accurate, precise, reproducible and linear within the range from 40.6% to 117.0% (Table 1, Figure 1) of the amount declared in the preparation (0.450 g in 0.6 g of tablet mass). The amount of paracetamol in TP–4 preparation determined by the cerometric method was 0.4623 ± 0.0020 g which satisfies the FP V demands (± 10% of the declared amount). The amount of cerium (IV) sulphate needed for titration of ascorbic acid and phenylephrine hydrochloride present in the weighed portion of TP–4 was 0.2 ml, which is equivalent to 1.91% of paracetamol content in this portion of TP–4. Therefore, it was assumed that in the quantitative composition of the preparation established by the producer, the cerometric method for paracetamol determination is selective.

Table 3. Determination of paracetamol and ascorbic acid in pharmaceutical preparations

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Determination, g</th>
<th>N</th>
<th>( \sigma )</th>
<th>( \sigma_i )</th>
<th>CV, %</th>
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<tbody>
<tr>
<td><strong>TP–4</strong></td>
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<tr>
<td>paracetamol 0.45 g</td>
<td>0.4623 ± 0.0020</td>
<td>6</td>
<td>0.00194</td>
<td>0.000794</td>
<td>0.42</td>
</tr>
<tr>
<td>ascorbic acid 0.030 g</td>
<td>0.0321 ± 0.0004</td>
<td>6</td>
<td>0.000429</td>
<td>0.000171</td>
<td>1.31</td>
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<tr>
<td>caffeine 0.025 g</td>
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<tr>
<td>phenylephrine, HCl 0.005 g</td>
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<tr>
<td><strong>Thomapyrin(^\text{®})</strong></td>
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<tr>
<td>acetylsalicylic acid 0.25 g</td>
<td>0.2052 ± 0.0017</td>
<td>6</td>
<td>0.00168</td>
<td>0.000687</td>
<td>0.82</td>
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<tr>
<td>paracetamol 0.20 g</td>
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<tr>
<td>caffeine 0.05 g</td>
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<tr>
<td><strong>Cefalgin(^\text{®})</strong></td>
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<tr>
<td>paracetamol 0.25 g</td>
<td>0.3306 ± 0.0061</td>
<td>6</td>
<td>0.00605</td>
<td>0.00247</td>
<td>1.83</td>
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<tr>
<td>propyphenesone 0.15 g</td>
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<tr>
<td>caffeine 0.050 g</td>
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<tr>
<td><strong>Panadol Extra</strong></td>
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<tr>
<td>paracetamol 0.5 g</td>
<td>0.4715 ± 0.0038</td>
<td>6</td>
<td>0.00365</td>
<td>0.00149</td>
<td>0.78</td>
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<tr>
<td>caffeine 0.065 g</td>
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<td><strong>Ring N</strong></td>
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<tr>
<td>acetylsalicylic acid 0.3 g</td>
<td>0.0245 ± 0.0007</td>
<td>6</td>
<td>0.000639</td>
<td>0.000261</td>
<td>2.61</td>
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<tr>
<td>caffeine 0.05 g</td>
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<tr>
<td>ascorbic acid 0.025 g</td>
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<tr>
<td><strong>Polopiryna C(^\text{®})</strong></td>
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<tr>
<td>acetylsalicylic acid 0.5 g</td>
<td>0.2003 ± 0.0035</td>
<td>6</td>
<td>0.00339</td>
<td>0.00138</td>
<td>1.69</td>
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<tr>
<td>ascorbic acid 0.2 g</td>
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<tr>
<td><strong>Vitaminum C</strong></td>
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<tr>
<td>ascorbic acid 0.2 g</td>
<td>0.1946 ± 0.0012</td>
<td>6</td>
<td>0.00123</td>
<td>0.000489</td>
<td>0.62</td>
</tr>
</tbody>
</table>

\(^a\) the cerometric method is non–selective
Satisfactory results of paracetamol determination were also obtained in Panadol Extra and Thomapyrin® (Table 3). An over estimated content of paracetamol in Cefalgin is a result of the presence of propyphenasone, 0.150 g, in a tablet of the average mass, so the cerometric method is non-selective and unsuitable for the determination of paracetamol in this preparation.

The titration method of ascorbic acid determination by a titrated solution of 2,6-dichloroindophenol is also based on a redox reaction. Analysis of the specificity of the method proved that the presence of phenylephrine hydrochloride, caffeine, acetylsalicylic acid, paracetamol and placebo does not interfere with the determination of ascorbic acid, because of a considerable difference in the redox potential of 2,6-dichloroindophenol (pH = 0, πc = +0.64 V; pH = 7, πc = +0.22 V) and cerium (IV) sulphate (πc = +1.44 V). Validation of the method performed for model mixtures of ascorbic acid and placebo proved that the method is accurate, reproducible and linear within the range from 52.4% to 140.0% (Table 2, Figure 2) of the amount declared in the preparation (0.030 g in 0.6 g of tablet mass).

The content of ascorbic acid in TP-4, Ring N, Polopiryna C®, Efferalgan Vitamine C and Vitamin C 0.2 satisfies the FP V demands (Table 3).

REFERENCES


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