Acetaminophen (N-acetyl-p-aminophenol) is widely used in antipyretic and analgesic therapy. This compound is generally perceived to be a safe agent, but chronic abuse or acute overdose can lead to hepatotoxicity [1]. The analytical methods to monitor acetaminophen are especially important in the neonatal and paediatric population, or in small animals where samples are often difficult to obtain, and the blood volume is limited. An easy way to quantify acetaminophen is based on a simple colorimetric test in which p-aminophenol, after an acidic hydrolysis step, is reacted with phenol in an alkaline medium to form an indophenol dye [2, 3]. Some of the published colorimetric methods utilize more than 0.5 ml of plasma or serum sample [3-6], so, laboratories that use analytical methods with limited plasma samples, could successfully perform the quantification of acetaminophen in paediatric population or in small laboratory animals. In this case, the sample volume should be as small as possible without causing damage to children, as a repeated blood sampling has been associated with neonatal anemia [7]. Previous researchers have reported, the spectrophotometric determination of some antihypertensive drugs in biological fluids using sulphonphthalein dyes [8], and the traces amount of acetaminophen in human plasma [9]. Our aim was to present an alternative micro method based on the indophenol reaction for the detection of acetaminophen in 200 µl of plasma sample. It provides a simple and economical screening test to be used in the laboratory.

The method was applied to the determination of acetaminophen in plasma samples arising from a pharmacokinetic study in rabbits. This animal model has been used for pharmacokinetic studies of antipsychotic agent [10]. In our work, New Zealand rabbits were employed, and plasma levels of acetaminophen were determined after administration of a rectal solution with the therapeutic dose of 100 mg/kg of the drug. Acetaminophen was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Methanol, 25% liquid NH₃, concentrated HCl, trichloroacetic acid, and phenol were analytical grade supplied by Merck-Mexico SA (Naucalpan, Mexico). The spectrophotometric apparatus was a Perkin Elmer UV/Vis Spectrometer Lambda 2S (Norwalk, CT, USA). The stock solution of acetaminophen (1 mg/ml) was prepared in methanol, and it was stored at 4 °C until use. Appropriate dilutions of acetaminophen were made in drug-free plasma to give final concentrations of 5, 10, 15, 20, and 25 µg/ml. The concentration range of 10–20 µg/ml corresponds to clinically associated levels with antipyresis, after a single rectal dose of 25 mg/kg [11, 12]. The procedure was adapted and validated from that reported by Frings and Saloom [3]. The determination consists in the reaction between p-aminophenol and phenol in an alkaline medium to form an indophenol dye, which exhibits a maximum extinction at 625 nm. The p-aminophenol is the hydrolysis product of acetaminophen and its metabolites, so by this way the total drug is quantified. Briefly, to an aliquot of heparinized plasma (200 µl of standard or sample), 200 µl of 20% trichloroacetic acid was added.

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The accuracy of the method was determined in plasma samples, containing acetaminophen with 5–25 µg/ml prepared by one person and assessed by another who had no knowledge of the concentrations. The samples were analyzed with respect to a standard calibration curve in plasma. The mean accuracy, 95% confidence limits (CI\_95\%), and its coefficient of variation (CV) were calculated. The analytical results were also compared in a graph, in which the determined vs. known concentrations were plotted. To test stability, the spiked blank plasma samples at three different concentrations (low, medium, and high) were made and were analyzed at the same time, after storing at −20 °C for 7 days. The CV of the slopes determined with reference to a standard calibration curve, obtained by plotting the absorbances vs. known plasma concentrations of acetaminophen, prepared the same day of the analysis [13, 14]. The method was applied in a pharmacokinetic study in rabbits. The animals were treated according to a protocol approved by the local Animal Ethics Committee (CICUAL-UAM-X-29), and following the Mexican federal regulations for the care and use of laboratory animals NOM-062-ZOO-1999 [15]. Six white male New Zealand rabbits (weight 3–4 kg) were used as an animal model. The animals fasted 12 h before the test. Before rectal administration, a 500 µl of blood sample was withdrawn from the marginal vein of each animal to serve as a control. At zero time, rabbits were treated with an aqueous solution of acetaminophen in a single dose of 100 mg. After administration, 500 µl of blood samples were taken at 0.25, 0.5, 1.0, 1.5, and 2 h and they were collected in heparinized polypropylene tubes. The plasma was separated by centrifugation and immediately frozen until their test according to the method described above. The pharmacokinetic parameters were computed by non-linear regression analysis using the WinNonlin version 2.1 (Parsight Corp. Palo Alto, CA, USA). The non-compartment model analysis was used to fit the in vivo data. Data are given as mean±standard error of the mean (SEM).

The analytical recovery was performed relating the absorbances of acetaminophen in plasma solutions, with respect to the absorbances of acetaminophen in aqueous solution of acetaminophen in a single dose of 100 mg. After administration, 500 µl of blood samples were taken at 0.25, 0.5, 1.0, 1.5, and 2 h and they were collected in heparinized polypropylene tubes. The plasma was separated by centrifugation and immediately frozen until their test according to the method described above. The pharmacokinetic parameters were computed by non-linear regression analysis using the WinNonlin version 2.1 (Parsight Corp. Palo Alto, CA, USA). The non-compartment model analysis was used to fit the in vivo data. Data are given as mean±standard error of the mean (SEM).

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The colorimetric method described in this paper allows the quantitative determination of acetaminophen in plasma samples of 200 µl. The method is simple and economic. The validation parameters evaluated in this work were within acceptable limits for clinical studies. The method was applied to the analysis of plasma samples obtained from a pharmacokinetic study in rabbits. The test is suitable for monitoring the plasma levels of acetaminophen, and it can be easily adapted by any hospital or clinical laboratory with non-special equipment.

Table 1: Pharmacokinetic parameters calculated with non-compartment model analysis after rectal administration of acetaminophen (100 mg) to rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (µg/ml)</td>
<td>39.24±6.05</td>
<td>MRT (h)</td>
<td>0.95±0.03</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>0.71±0.14</td>
<td>λ_1 (h^{-1})</td>
<td>0.64±0.08</td>
</tr>
<tr>
<td>AUC_Ce_a (µg/mlh)</td>
<td>55.78±9.81</td>
<td>t_1/2 (h)</td>
<td>1.21±0.20</td>
</tr>
</tbody>
</table>

AUC: area under the curve, MRT: mean residence time, mean±SEM, n = 6

Some pharmacokinetic parameters calculated in this work agree with those reported by other researchers, e.g. the C\_max value of 39.24±6.05 µg/ml with the C\_max value of 25 µg/ml found after rectal administration of a commercial suppository with 100 mg of acetaminophen to rabbits [16]. Although it is the same dose used by us, our data is slightly higher due to acetaminophen was administered in an aqueous solution.

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**CONFLICT OF INTERESTS**

Declared none

**REFERENCES**


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