Development and application of a portable instrument for drugs analysis in pharmaceutical preparations

Paulo Roberto da Silva Ribeiro¹,*, Helena Redigolo Pezza², Leonardo Pezza², Liliane Spazzapam Lima², Matthieu Tubino³

¹Centre for Social Science, Health and Technology, Federal University of Maranhão, Imperatriz, MA, Brazil, ²Institute of Chemistry, São Paulo State University, UNESP, Araraquara, SP, Brazil, ³Department of Analytical Chemistry, Institute of Chemistry, State University of Campinas, UNICAMP, Campinas, SP, Brazil

This article describes the application and performance of an inexpensive, simple and portable device for colorimetric quantitative determination of drugs in pharmaceutical preparations. The sensor is a light detector resistor (LDR) incorporated into a black PTFE cell and coupled to a low-cost multimeter (Ohmmeter). Quantitative studies were performed with captopril/p-chloranil/H₂O₂ and methyldopa/ammonium molybdate systems. Calibration curves were obtained by plotting the electrical resistance of the LDR against the concentration of the colored species in the ranges 1.84 × 10⁻⁴ to 1.29 × 10⁻³ mol L⁻¹ and 5.04 × 10⁻⁴ to 2.52 × 10⁻³ mol L⁻¹ for captopril/p-chloranil/H₂O₂ and methyldopa/ammonium molybdate systems, respectively, exhibiting good coefficients of determination. Statistical analysis of the results obtained showed no significant difference between the proposed methodologies and the official reported methods, as evidenced by the t-test and variance ratio at a 95% confidence level. The results of this study demonstrate the applicability of the instrument for simple, accurate, precise, fast, in situ and low-cost colorimetric analysis of drugs in pharmaceutical products.

INTRODUCTION

Captopril, 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline (Figure 1), (CPT) is an angiotensin-converting enzyme inhibitor, which reduces peripheral resistance and lowers blood pressure. It is extensively used for the treatment of hypertension (Florey, 1988) and congestive failure (Brunton, Chabner, Knollman, 2010).

![Chemical structure of captopril.](image1)

In order to assure the quality of CPT containing pharmaceutical formulations, several analytical techniques have been used for determination of the drug, including batch fluorimetry (Hormozi-Nezhad et al., 2013), chemiluminescence (Zhang et al., 2009), high-performance liquid chromatography (Karakosta, Tzanavaras, Themelis, 2012), electrochemical methods (Jamali, Karimi-Maleh, Khalilzadeh, 2014; Ensafi et al., 2011; Karimi-Maleh, Ensafi, Allafchian, 2010; Khalilzadeh et al., 2010), volumetric titration (USP, 2007), diffuse reflectance spectroscopy (Ribeiro; Pezza; Pezza, 2011) and spectrophotometry (Gouda, Amin, 2010; El-Didamony, Erfan, 2010; Ribeiro, Pezza, Pezza, 2005; Ribeiro, 2005) and diffuse reflectance spectroscopy (Ribeiro, Pezza, Pezza, 2006).

However, some of these methods are inherently expensive, time consuming, laborious and not well suited for process control monitoring. In addition, chromatographic methods are slow and require expensive and complicated instrumentation, features rendering them unattractive for routine analysis. The titrimetric method can suffer from a lack of specificity and sensitivity under certain circumstances, such as in the presence of unsaturated organic compounds. The proposed approach is relatively reliable, low cost and sufficiently compact to be applied even by untrained personnel. Thus, there is considerable interest in the development of highly reliable, cost effective, sensitive, and selective detection devices for determination of CPT and MTD in marketed dosage formulations.

Instrumentation has become integral to chemical measurements. A multimeter is an essential piece of equipment on an electrician’s workbench. It is a universal instrument, useful not only for measuring electrical quantities, such as voltage, current, and resistance, but also for testing electrical and electronic circuits.

Thus, the multimeter could also be a very useful instrument for the chemistry laboratory bench. Contemporary electronics and instrumentation have reached a level of application that allows the measurement, direct or indirect, of chemical quantities. These measurements involve the encoding of chemical information into electrical signals. This transduction can be carried out through a variety of physical and chemical principles, and in most cases, the resultant electrical quantity can be measured using a multimeter. Colorimetric (Acharya, Jayaraman, 1995; Richter, Rocha, Angnes, 2004; Rossi, He, Tubino, 2000; Wang, 2000), potentiometric (Barnard, 1968; Ooba, 1991; Richter, 2003; Sevilla, Alfonso, Andres, 1993; Vreman, 1994; Watanabe, 1997) and conductometric (Sevilla, Alfonso, Andres, 1993; Yue, Ngin, Hailin, 1996) systems based on a multimeter have been described in the literature.

Coupled with optical sensors and transducers, the multimeter could constitute a means of measuring chemical parameters. Optical sensors are of great interest because of their favorable characteristics when compared
to other kinds of sensors. Portable optical sensors in particular are useful for enabling *in situ* chemical analysis (Arnold, 1992; Seitz, Sepaniak, 1988; Wang, 2000).

Moreover, optical sensor-based instruments are simple to assemble, low cost, versatile, and can be employed in the determination of many chemical parameters with promising results. Thus, the objective of this study was to describe the application of a portable instrument for determination of CPT and MTD in pharmaceutical formulations. The results showed good concordance with those obtained by the USP standard procedure (USP, 2007) for CPT and Brazilian Pharmacopoeia standard procedure (Brasil, 2010) for MTD, at a 95% confidence level.

**MATERIAL AND METHODS**

**Apparatus**

A prototype of the portable device (Figures 3A and 3B) was used for all resistance measurements performed in this study (Rossi, He, Tubino, 2000). A 9 W Startec desk lamp was used as the light source, placed at a distance of around 17.0 cm from the cell. A Minipa® ET-1502 digital multimeter was used to collect the resistance measurements. An ordinary glass tube with internal diameter of 5.0 mm and length of 50.0 mm was used as the sample cell.

![FIGURE 3 - (A) General view of optical device mounted on digital multimeter. (B) Schematic diagram of optical device.](image)

Volume measurements were made with a plunger-operated pipetter (100-1000 µL) and Metrohm model 665 automatic burettes. All experiments were performed in a thermostatically-controlled room (25±1) °C.

**Reagents and solutions**

All reagents and chemicals used were analytical grade and solvents were spectroscopic grade. Deionized water was used for the preparation of solutions and samples.

**Captopril/p-chloranil/H₂O₂ system**

CPT (reference substance) was purchased from Purifarma, São Paulo, Brazil (purity grade > 99.9%, calculated on dried basis). CPT characteristics were consistent with the United States Pharmacopoeia (USP) (USP, 2007). A stock solution (CPT₀ = 4.65 × 10⁻³ mol l⁻¹) of CPT standard was prepared daily by dissolving 10.0 mg of the reference substance in methanol and diluting to the mark in a 10.0 mL volumetric flask. Working standard solutions were obtained by appropriate dilution of the stock solution with the same solvent and standardized using the standard procedure reported in the official method of the USP (USP, 2007).

p-Chloranil (Sigma-Aldrich) solution (0.5% w/v) was prepared daily by dissolving 125.0 mg in 25 mL of 1,4-dioxane (Mallinckrodt - p.a. grade).

H₂O₂ 10.35 mol L⁻¹ (Merck) was standardized (Vogel, 1986) by titration with potassium permanganate and utilized in the experiments.

Pharmaceutical formulations (tablets) of four commercial brands were analyzed. The tablets were purchased from local drugstores and all were tested prior to the listed expiration date. All pharmaceuticals studied were package labeled as containing 25.0 mg of CPT per tablet.

**Methyldopa/ammonium molybdate system**

Stock 4.65 x 10⁻³ mol L⁻¹ MTD (Sigma, St. Louis, MO, USA, 99.95%) solution was prepared daily by dissolving 50.0 mg of the drug in 50.0 mL of deionized water. Using a mechanical shaker, the powder completely disintegrated after shaking for 15 minutes. Working standard solutions were obtained by appropriate dilution of this stock solution with the same solvent and were standardized by the standard procedure reported in Brazilian Pharmacopoeia (Brasil, 2010).

Ammonium molybdate [(NH₄)₆Mo₇O₂₄·4H₂O] was purchased from Merck (Darmstadt, Germany, p. a.). The ammonium molybdate aqueous solution 1.0% (m v⁻¹) was prepared daily.

Pharmaceutical formulations (tablets) of four commercial brands were analyzed. These tablets were purchased from local drugstores and all were tested prior to the listed expiration date. All pharmaceuticals studied were package labeled as containing 250 and 500 mg of MTD per tablet.
Methodology

Captopril/p-chloranil/H$_2$O$_2$ system

- Procedure for calibration curve
  The calibration curve was constructed by transferring 800 µL of CPT working standard solutions (comprising 1.84 x 10$^{-4}$ to 1.29 x 10$^{-3}$ mol L$^{-1}$ of this compound) into each series of 5.0 mL standard flasks. A total of 900 µL of $p$-chloranil solution (0.5% w v$^{-1}$), 225 µL of H$_2$O$_2$ 10.35 mol l$^{-1}$ and 2 mL of methanol was added to each standard flask and left to stand at 50 °C for 30 min (Ribeiro, 2011). The blank solution was prepared in a similar manner, but omitting the CPT. A constant volume of 1200 µL of this solution was introduced into the sample cell with micropipette and the resistance measurements were obtained against the corresponding reagent blank. Calibration graphs were prepared by plotting the electrical resistance of the LDR against drug concentration. The graphs, or the corresponding linear least squares equations, were used to convert the resistance measurement into CPT concentration for any given sample analyzed.

- Procedure for assay of CPT in pharmaceutical samples
  Four commercial brands of CPT tablets (A, B, C and D) sourced from local drugstores were randomly selected and analyzed using the portable electrochemical device. For the determination of CPT in pharmaceutical samples, twenty tablets were weighed to calculate the average tablet weight. Tablets were finely powdered and homogenized. A portion of this powder, equivalent to ca. 10.0 mg of CPT, was accurately weighed and dissolved in 7 mL of methanol by shaking for 15 min on a mechanical shaker. The resulting mixture was filtered through Whatman filter paper No. 41 into a 10 mL volumetric flask. The filter paper was washed using solvent and filtrate. The washing was subsequently transferred to a volumetric flask and the volume made up to the mark. Aliquots of 1000 µL from this solution were transferred into 5.0 mL graduated flasks and analyzed according to the recommended procedure for the calibration curve. The quantity per tablet was calculated from the standard calibration graph.

Methyldopa/ammonium molybdate system

- Procedure for calibration curve
  A volume of 1000 µL of MTD working standard solutions was transferred into each series of 5.0 mL standard flasks, comprising 5.04 x 10$^{-4}$ to 2.52 x 10$^{-3}$ mol L$^{-1}$ of the drug. A total of 1000 µL 2.0% ammonium molybdate was added to each graduated flask and the volume completed with deionized water (Ribeiro, 2005). The blank solution was prepared in a similar manner, but omitting the MTD. A constant volume of 1200 µL of this solution was introduced into the sample cell with micropipette and resistance measurements were obtained against the corresponding reagent blank. Calibration graphs were prepared by plotting the electrical resistance of the LDR against drug concentration. These graphs, or the corresponding linear least squares equations, were used to convert resistance measurement into captopril concentration for any given sample analyzed.

- Procedure for assay of MTD in pharmaceutical samples
  Four commercial brands of MTD tablets (E, F, G and H) sourced from local drugstores were randomly selected and analyzed using the portable electrochemical device. The average tablet weight was calculated from the contents of 20 tablets. Tablets were finely powdered and weighed. A portion of this powder, equivalent to ca. 200.0 mg of MTD, was accurately weighed and dissolved in 80 mL of water by shaking for 15 min on a mechanical shaker. The resulting mixture was transferred into 100.0 mL graduated flasks and the volume completed with deionized water. This solution was clarified by passing it through a cotton column filter, rejecting the first 20 mL. Aliquots containing equivalent to 1.51 x 10$^{-3}$ mol L$^{-1}$ were transferred into 5.0 mL graduated flasks and analyzed according to the recommended procedure for the calibration curve. The quantity per tablet was calculated from the standard calibration graph.

Repeatability study (precision)

To examine the repeatability of the measurement system, replicate $(n = 10)$ determinations were made for the solutions (samples C and F) containing equivalent to 7.36 x 10$^{-4}$ mol L$^{-1}$ of CPT and 1.51 x 10$^{-3}$ mol L$^{-1}$ of MTD, respectively. These solutions were analyzed according to the recommended procedures for the assay of CPT and MTD content in pharmaceutical formulations. The precisions were calculated in terms of percentage relative standard deviation (% RSD).

Accuracy/recovery studies

To study the accuracy of the proposed device for the determination of CPT and MTD in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed...
by addition of known amounts ($1.66 \times 10^{-4}$; $1.84 \times 10^{-4}$; $2.02 \times 10^{-4}$ and $2.21 \times 10^{-4}$ mol L$^{-1}$ of CPT and $4.54 \times 10^{-4}$; $5.04 \times 10^{-4}$ and $6.05 \times 10^{-4}$ mol L$^{-1}$ of MTD, corresponding to levels of 90; 100; 110 and 120%, of these drugs, respectively) of the standard substance (pure drugs) to a known concentration of the previously analyzed commercial tablets (CPT: samples A, B, C and D - MTD: samples E, F, G and H). The resulting mixtures were analyzed according to the recommended procedure for the assays of CPT and MTD content in pharmaceutical formulations. Drug recovery was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drugs.

RESULTS AND DISCUSSION

Colorimetry involves the measurement of the concentration of chemical species through the amount of light absorbed at certain wavelengths. The extent of absorption of light is measured based on the intensity of the radiation transmitted by the sample solution (Holler, 2009).

A multimeter can perform colorimetric measurements when coupled with a photodetector, such as a light-dependent resistor (LDR). The LDR is a semiconductor whose resistance depends on the intensity of the radiation striking its surface. The value of the resistance of the sensor (LDR) decreases as the intensity of the incident light increases (Rossi, He, Tubino, 2000). Thus, for quantitative purposes, calibration curves can be obtained by plotting the electrical resistance of the LDR (R) against the analyte concentration (C) by a linear relationship. In the present study, a multimeter was used to measure the resistance of the LDR.

Captopril/p-chloranil/H$_2$O$_2$ system

CPT is a nitrogenous compound that acts as $n$-donors to the $\pi$-acceptors. These acceptors react with the basic nitrogenous compounds to form charge transfer complexes or radical anions according to the polarity of the solvent used (Bebawy, 1999). Hence $p$-chloranil was used in this stage of work as the reagent for the determination of CPT in the presence of hydrogen peroxide. The addition of H$_2$O$_2$ to the reaction medium increases the reaction rate (Sawyer, 1991; Mattos et al., 2003). The optimum experimental conditions and studies of the stability of this product were established in a study previously conducted at our laboratory (Ribeiro, 2011). Thus, the experimental conditions used in the present study were the same as those utilized in the previously tested approach.

The relationship between the electrical resistance of the LDR and the concentration of CPT under optimal conditions was examined. The analytical curve (Figure 4) was obtained by the method of least squares from eleven points, each of which was the average of three determinations. This curve was obtained by plotting the electrical resistance of the LDR against the CPT concentration over the range $1.84 \times 10^{-4}$ to $1.29 \times 10^{-3}$ mol L$^{-1}$ of CPT in the final solution, exhibiting a good coefficient of determination ($R^2 = 0.9946$; slope $= 1871.0 \pm 61.5$ L mol$^{-1}$ cm$^{-1}$ and intercept $= 0.181 \pm 0.033$). The limit of detection (3.SD$_{blank}$) (Long, Winefordner, 1983) was $1.54 \times 10^{-5}$ mol L$^{-1}$ of CPT under the experimental conditions described.

Methyldopa/ammonium molybdate system

The reaction of MTD with molybdate ions produces a colored water soluble complex (Ribeiro, 2005). The absorption spectrum of this reaction product shows
that the best analytical wavelength is located at 410 nm. The optimum experimental conditions and studies of the stability of this product were established in a study previously conducted at our laboratory. Thus, the experimental conditions used in the present study were the same as those utilized in the previous study.

The analytical curves were obtained by the method of least squares from eleven points, each of which was the average of three determinations. This curve (Figure 5) was obtained by plotting the electrical resistance of the LDR against the MTD concentration over the range $5.04 \times 10^{-4}$ to $2.52 \times 10^{-3}$ mol L$^{-1}$ of MTD in the final solution, exhibiting a good coefficient of determination ($R^2 = 0.9980$; slope $= 1397.2 \pm 28.0$ L mol$^{-1}$ cm$^{-1}$ and intercept $= 0.188 \pm 0.035$). The limit of detection (3. SD$\text{blank}$) (Long; Winefordner, 1983) was $7.99 \times 10^{-5}$ mol L$^{-1}$ of MTD under the experimental conditions described. On the test of linearity by the ANOVA lack of fit model, the calculated value of $F_{\text{cal}}$ for the analyte was less than the tabulated $F$ value ($F_{\text{tab}}$ (5,14) = 2.96), indicating linearity of response.

**Analytical applications and repeatability studies**

In order to assess the utility of the present portable device, it was applied to provide an estimation of CPT and MTD in commercial tablets. The samples were prepared and analyzed according to recommended procedures. The measurement system was then successfully applied for drug determination in four tablet formulations of these drugs. The results, presented in Table I, compare favorably with the official methods of the USP for CPT (USP, 2007) and of the Brazilian Pharmacopoeia for MTD (Brasil, 2010), attesting to the applicability of the proposed device for the determination of CPT in pharmaceutical dosage forms.

Comparing the results obtained using the proposed device against those obtained by the official methods, based on the Student’s $t$ values (accuracy) and on the $F$ test (precision) concordance was observed for the 95% ($\alpha = 0.05$) confidence level (Miller; Miller, 1993). Thus, both Student’s $t$ and $F$ tests showed statistical equivalence between the results of the proposed device and the official methods, thereby attesting to the applicability of the proposed device for the determination of drugs in pharmaceutical dosage forms.

The RSD values obtained using the proposed device for the samples ranged from 0.4 to 1.9% for CPT and 0.4 to 2.2% for MTD, as shown in Table I. According to Horwitz (1982), the maximum RSD value acceptable

**TABLE I - Results of CPT and MTD assays in tablets**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Label value$^a$</th>
<th>Proposed portable device</th>
<th>Official method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found$^b$</td>
<td>RSD (%)$^c$  $t$-value</td>
<td>$F$-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.78)$^d$</td>
<td>(19.00)$^d$</td>
</tr>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>

**Determination of CPT in commercial pharmaceutical preparations**

| A | 25.0 | 25.4±0.1 | 0.4 | 2.07 | 1.00 | 24.9±0.1 | 0.4 |
| B | 25.0 | 25.6±0.2 | 0.8 | 2.52 | 1.00 | 24.6±0.2 | 0.8 |
| C | 25.0 | 25.4±0.2 | 0.8 | 2.10 | 1.00 | 24.8±0.2 | 0.8 |
| D | 25.0 | 25.8±0.5 | 1.9 | 1.59 | 2.78 | 25.2±0.3 | 1.2 |

**Determination of MTD in commercial pharmaceutical preparations**

| E | 250.0 | 257.6 ± 1.0 | 0.4 | 2.05 | 1.23 | 256.3 ± 0.9 | 0.4 |
| F | 250.0 | 254.0 ± 1.5 | 0.6 | 2.69 | 1.14 | 258.3 ± 1.6 | 0.6 |
| G | 500.0 | 510.1 ± 1.0 | 0.2 | 0.19 | 3.24 | 510.3 ± 1.8 | 0.4 |
| H | 500.0 | 507.5 ± 1.1 | 2.2 | 0.80 | 1.60 | 513.9 ± 9.0 | 1.7 |

$^a$Label for content of tablets: mg unit$^{-1}$. $^b$Average value ± standard deviation (SD) of three determinations. $^c$Relative standard deviation (RSD) of three determinations. $^d$Figures between parentheses are theoretical values of $t$ and $F$ at $P = 0.05$
for the working level of the analyte \((1.15 \times 10^{-3} \text{ mol l}^{-1})\) is 8.0%. The AOAC (1993) set the maximum acceptable RSD value at 5.3% for the same analyte level.

The repeatability of the measurement system was investigated. The relative standard deviations (RSDs) were 1.0% and 1.6% for solutions \((n = 10)\) containing CPT and MTD, respectively. These results are evidence of good repeatability of the measurement system for drugs analysis.

### Accuracy/recovery studies

To ascertain the accuracy and precision of the portable device, recovery studies were carried out \((n = 3)\) by spiking different concentrations of pure drugs in the pre analyzed samples with four different concentrations of standards within the analytical concentration range of the proposed method. The results of the recovery tests are given in Table II. The recovery (mean values) for

<table>
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<th>Drug</th>
<th>Sample</th>
<th>Added (x 10^4 mol l^-1)</th>
<th>Found (x 10^4 mol l^-1)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
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<td>CPT</td>
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<td>1.66</td>
<td>1.67</td>
<td>100.6</td>
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<tr>
<td></td>
<td></td>
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<td>1.85</td>
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<td>µ ± = 99.92±1.68</td>
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</table>

*Mean ± relative standard deviation (RSD) of three determinations*
samples within the 99.62-100.9% range and RSDs within 0.52-1.43% for CPT and within the 99.48-100.3% range and RSDs within 0.63-1.68% for MTD, confirmed an accurate and precise measurement system for application to pharmaceutical dosage forms.

CONCLUSION

Based on the above results and from general observations in the laboratory, it can be concluded that the very simple and portable device proposed by Rossi, He, Tubino, (2000) can be used for colorimetric quantitative determination of drugs in pharmaceutical preparations. Statistical comparison of the results for the portable device against those for the official reported methods indicated no significant difference in accuracy and precision at a 95% confidence level. Additionally, the device fulfills all the main requirements of routine analysis as it is robust and has low instrumentation and operational cost in comparison to chromatographic methods.

Thus, the results obtained demonstrate clearly demonstrate the potential alternative use of this measurement system for the quality control of drugs. Moreover, this instrument has applicability for simple, accurate, precise, fast, in situ and low-cost colorimetric analysis of drugs in pharmaceutical products.

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