Simultaneous assay of codeine phosphate and diphenhydramine hydrochloride in cough mixtures by zero-order derivative UV spectrophotometry

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This study evaluated a zero-order derivative UV spectrophotometric method for the simultaneous determination of binary components, codeine phosphate and diphenhydramine HCl in cough mixtures. The method was based on the measurement of zero-order derivative amplitudes at isosbestic points, 258 and 264 nm, for the assay of codeine phosphate and diphenhydramine HCl (0.05 to 0.15 mg/ml). The method does not require any pretreatment or the use of dyes or complexing agents. The scanned spectrum for diphenhydramine HCl standard in 0.1 M HCl showed absorptions at 252 nm and 258 nm ($\lambda_{\text{max}}$) while codeine phosphate standard in 0.1 M HCl solution showed maximum absorptions at 284 nm. However, both spectra showed an overlap at 258 nm and 264 nm. The accuracy and precision of the method was evaluated by performing five replicate analyses. The relative standard deviation (rsd) (%) for both codeine phosphate and diphenhydramine HCl ranged between 2.64 and 8.85% while the standard errors of mean concentrations were below 10 and 0.5%, respectively. The 95% confidence interval presented for students t-test is suggestive of a satisfactory precision and accuracy of the method used. The mean percentage recovery of both drugs from the spiked formulated cough mixtures ranged between 96.99-102.40% and 98.07-101.56% for codeine phosphate and diphenhydramine HCl, respectively. The technique in this study when compared with the BP (2008) method and specification for single dosage forms for codeine phosphate (95.0–105.0%) and diphenhydramine HCl (90.0–110.0%) could be of good application in simple analytical assays of such binary components in mixtures.

Key words: Codeine phosphate, diphenhydramine HCl, derivative UV-spectrophotometry, binary components, mixtures.

INTRODUCTION

Codeine phosphate (COP) and Diphenhydramine hydrochloride (DPH) are commonly used in preparation of cough mixtures either in single or combined dosage forms as cough expectorants or suppressants. They are known to act synergistically to produce the desired therapeutic effect. Codeine is a derivative of morphine, a naturally occurring alkaloid obtained from opium and other poppy saps such as Papaver bracteatum and Papaver somniferum (Charlton, 2005). Currently, it is mostly obtained by synthesis and readily available in salt forms as hydrochloride or phosphate. It is useful in the suppression of cough reflex and for the treatment of mild to moderate opioid-sensitive pains (Moore et al., 1997). Codeine is chemically known as 7, 8-didehydro-5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol phosphate hemihydrates as shown in Figure 1. Diphenhydramine, an over-the-counter (OTC) antihistamine, sedative and hypnotic is used to treat allergies, motion sickness, insomnia, cough, nausea and phenothiazine drug induced abnormal muscle movement (Carr et al., 1985; Charlton, 2005). Diphenhydramine is an isomer of phenyltoloxamine and it is chemically known as 2-(diphenylmethoxy)-N,N-dimethylethanamine hydrochloride (Figure 1).

Derivative spectrophotometry is an advanced modern spectrophotometric technique, which allows for extracting
both qualitative and quantitative information from spectra with overlapped bands. The basic principle has to do with the utilization of the first- or higher-order derivatives of absorbance while relying on the wavelength from parent zero-order (Ojeda et al., 2004).

The application of derivative spectrophotometry in pharmaceutical analysis has been critically reviewed with a number of reports on analytical methods for the assay of these drugs separately or simultaneously (Shamsa and Maghssoudi, 1976; Erdal et al., 2002; Karpińska, 2004; Duong et al., 2009), non-aqueous capillary electrophoresis, HPLC (Hood and Cheung, 2003; Barbas et al., 2000), gas chromatography (Tonn et al., 1993) for single, binary and multiple dosage forms. However, most of these techniques require the use of expensive instruments, dyes and/ or reagents in spite of the fact that the derivatisation can lead to the separation of unresolved signals and reduction of spectral background interferences (Hood and Cheung, 2003; Yuming et al., 2005; Chunling et al., 2006). In this study, the technique permits the quantification of COP in the presence DPH without initial separation or purification using a simple and inexpensive zero-order derivative spectrophotometry. The method is comparable to BP official methods for single component preparations of COP and DPH as there are no official compendia methods for the simultaneous analysis of the combination of these drug substances. In particular, this technique was used in this study to determine the percent purity of COP and DPH in four different brands of cough mixtures in multi-component pharmaceutical formulations.

**MATERIALS AND METHODS**

Four brands of cough mixtures containing codeine phosphate and diphenhydramine HCl as actives were purchased from retail pharmacies in Yenagoa, Bayelsa State, Nigeria and are coded A to D. Their brand names, manufacturers, dates of manufacture, expiry dates, batch numbers and NAFDAC Number (Product Regulatory Authority Registration Number) were duly recorded. Codeine phosphate and diphenhydramine HCl reference standards were obtained from Afrab Chemicals Ltd (Lagos, Nigeria). Analytical grade reagents such as, diethyl ether, hydrochloric acid and sodium hydroxide were sourced from BDH Ltd Poole England.

**Instrumentation and measurements**

A Thermo-Spectronic single beam UV-visible spectrophotometer inter-phased to a computer with version 32 software and 1 cm quartz cuvette was used for scanning to determine \( \lambda_{\text{max}} \) for COP and DPH reference standards individually and in combination and also for the measurement of absorbance of extracted drug products. A digital pH meter and pycnometer (50 ml) were used, respectively, to determine pH and weight per ml of drug products at 25°C. All instruments were calibrated before use.

**Preparation of standard solutions**

1 mg/ml primary stock solutions of COP and DPH were prepared by accurately weighing 0.1 g of each standard and dissolving separately in 100 ml volumetric flask using 0.1 M HCl. The percentage purity of codeine phosphate standard used was corrected by using \((0.1\times100/99.8)\) g as a correction factor.

**Physicochemical analysis**

Physicochemical properties such as pH, weight per ml at 25°C, average filled volume were determined using pH meter, pycnometer (50 ml) and measuring cylinder (100 ml), respectively. The pH meter was calibrated using pH buffer 4, 7 and 9 (Table 2).

**Determination of \( \lambda_{\text{max}} \) for COP and DPH**

Separate solutions of COP and DPH standards in 0.1M HCl were scanned between 200 to 350 nm of the UV-region to ascertain possible wave length of absorptions. Prior to this, standard calibration curves were made for both COP and DPH.

**Simultaneous extraction and analysis of drug samples**

The method used for extraction is a modified BP official method (1998) for COP as a single component in syrups. 20 g of drug sample was weighed at 25°C and transferred into a separatory funnel. Sample was acidified with 10 ml 2M HCl and shaken with 3
Table 1. Physicochemical parameters of the four different brands of cough mixtures containing codeine phosphate and diphenhydramine hydrochloride.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cough mixtures brand codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>A</td>
</tr>
<tr>
<td>Description</td>
<td>Dark red</td>
</tr>
<tr>
<td>pH</td>
<td>5.5</td>
</tr>
<tr>
<td>wt/ml (g/ml)</td>
<td>1.3006</td>
</tr>
<tr>
<td>Average filled volume (ml)</td>
<td>96.95±2.76</td>
</tr>
<tr>
<td>Percentage deviation (%)</td>
<td>2.85</td>
</tr>
</tbody>
</table>

× 15 ml of diethyl ether in a fume hood. The ethereal layer was discarded. The aqueous phase was then basified with 10 ml of 5 M NaOH and extracted with 15 ml of ether for 10 successive times. The combined ethereal extract was washed with 2 × 5 ml of distilled water and the combined aqueous washing was shaken with 15 ml of ether. The pooled ethereal extract was evaporated to 1 to 2 ml on a steam bath and to dryness using gentle stream of nitrogen gas. Residue was re-dissolved with 20 ml of 0.1 M HCl and made to 100 ml in a volumetric flask using the same 0.1 M HCl. An aliquot of 20 ml was diluted to 50 ml in a volumetric flask using same 0.1M HCl. Absorbance readings were measured at 258 and 284 nm.

The simultaneous determination of COP and DPH by UV spectrophotometry could be expressed by the following simultaneous equations (Olaniyi, 2000; Christian, 2004; Mendham et al., 2006);

\[
A_1 = E_{X1}C_X + E_{Y1}C_Y
\]

\[
A_2 = E_{X2}C_X + E_{Y2}C_Y
\]

Where:

\( A_1 \) and \( A_2 \) are absorbance at 258 and 284 nm, respectively.\n
\( C_X \) and \( C_Y \) are concentrations of COP and DPH, respectively.\n
\( E_{X1} \) and \( E_{Y1} \) are molar absorptivities of COP and DPH, respectively, at 258 nm.\n
\( E_{X2} \) and \( E_{Y2} \) are molar absorptivities of COP and DPH, respectively, at 284 nm.

Precision and recovery of the analytical method

The accuracy and precision of the method was evaluated by performing five replicate analyses on the reference standard of COP and DPH.

Analytical parameters

To select appropriate working concentrations, the limit of detection (LOD) and limit of quantification (LOQ), were determined by making a standard calibration curve separately for diphenhydramine HCl and codeine PO_4. Linearity of prepared concentrations and recorded absorbance at 258 and 284 nm for diphenhydramine HCl and codeine PO_4 reference standards, respectively, were analysed. These concentrations were made based on the range of the label claim of drug substances, which is the ethereal extract of formulated drugs.

Statistical analysis

The student’s t-test of the Graphpad prism software version 2 was employed to calculate the 95% confidence interval of precision and accuracy of the analytical method with p < 0.05 as the level of significance.

RESULTS

The standard filled volume for all the brands ranged between 92.3–100.5 ml. The individual filled volumes and standard deviations as well as the percentage deviation of the filled volume are as shown in Table 1. All the brands were acidic and viscous with pH values ranging from 5.00 to 6.80 while weight per millilitre was between 1.30 and 1.37. Table 2 summarizes some statistical data obtained for COP and DPH from the technique.

The intra-day precision (repeatability) of this technique was evaluated by analyzing six replicates of four sample mixtures coded A to D containing simultaneously codeine phosphate and diphenhydramine hydrochloride. The inter-day precision (intermediate precision) of this technique was also evaluated with these four mixtures being analyzed during five consecutive days as shown in Table 3. To evaluate, the accuracy of this technique, recovery studies were carried out by the standard addition method. For this, known quantities of pure COP and DPH equivalent to 80, 100 and 120% of their label claim in mixtures were mixed with corresponding definite amounts of pre-analyzed formulations such that final concentrations of the drugs were within the techniques’ calibration range and the mixtures were analyzed as before. The total amount of each drug was then determined and the drug added amount was calculated by difference (Table 4). The mean percent recoveries of both drugs from the spiked formulated cough mixtures were quantitative ranging between 96.99 to 102.4% and 98.07 to 101.56% for COP and DPH, respectively, indicating the techniques’ good accuracy.

The scanned UV spectrum for DPH standard in 0.1 M HCl solution showed absorptions at 252 and 258 nm with maximum at 258 nm while COP absorbed maximally at 284 nm, which is in agreement with BP 2008 specifications as shown in Figure 2. Also, spectra analysis of both the combined standards and the ethereal
Table 2. Analytical parameters for simultaneous spectrophotometric method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Codeine phosphate</th>
<th>Diphenhydramine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum wavelength (λ_{max}) nm</td>
<td>284</td>
<td>258</td>
</tr>
<tr>
<td>Limit of detection (LOD) mg/mL</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Limit of quantification (LOQ) mg/mL</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Linearity (working range) mg/mL</td>
<td>0.050–0.15</td>
<td>0.050–0.45</td>
</tr>
<tr>
<td>Coefficient of correlation, r</td>
<td>0.9988</td>
<td>0.9926</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.3073</td>
<td>0.1099</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>5.7140</td>
<td>1.9885</td>
</tr>
<tr>
<td>Regression equation, y = a + bx</td>
<td>a + bx</td>
<td>a + bx</td>
</tr>
</tbody>
</table>

Regression equation, y=a + bx. Where, y=absorbance, a=intercept, b=slope, x=concentration (mg/ml)

Extracts of formulated drugs in 0.1 M HCl solution were compared and both absorption peaks were similar as shown in Figure 3. The ethereal extract of all drug product in 0.1M HCl, showed very strong absorption at 284 nm with a medium intensity at 258 nm being maxima absorption wavelengths for COP and DPH in 0.1 M HCl, respectively.

**DISCUSSION**

The suitability and validity of this technique was examined as to detection and quantification limits, precision (reported as relative standard deviation, RSD), linearity (evaluated by regression equation) and accuracy (reported as percentage recovered). The linearity range was confirmed to be between 0.001 mg/ml and 0.15 mg/ml for the combined standards. The lower limit of 0.001 mg/ml corresponds to the limit of detection (LOD) for both COP and DPH combination with respect to preparations under consideration. The lower limit of quantification (LLOQ) was 0.05 mg/ml, corresponding to a fifty-fold increase in concentration with respect to the detection limit for both standards. Thus, the working quantification range for the proposed simultaneous UV method is 0.05 to 0.15 mg/ml concentration of COP and DPH. Spectra analysis of the combined standards and that of the ethereal extracts of formulated drugs in 0.1 M HCl were examined and both absorption peaks and wavelengths were found to be identical. The correlation coefficient (R) for the calibration curves were 0.9988 and 0.9963 for codeine PO_4 and diphenhydramine HCl, respectively. Regression equations, y = 0.3073 + 5.7140x and y = 0.1099 + 1.9885x, respectively for COP and DPH were in line with the Beer-Lambert equation.

The intra-day and inter-day precision of this technique was such that samples B to D compare to the innovator sample A showed a good precision (RSD < 10%) of the analytical technique. The recoveries of both drugs from the spiked formulated cough mixtures were quantitative indicating the techniques' good accuracy. The method developed in this study was successfully applied to the
Table 4. Mean percentage recovery of COP and DPH from sample mixtures with UV derivative spectrophotometric technique.

<table>
<thead>
<tr>
<th>Sample mixture code</th>
<th>Analyte</th>
<th>Theoretical concentration (mg/5 ml)</th>
<th>Mean concentration of analyte recovered (mg/5 ml) (n = 3)</th>
<th>% recoveries for analyte in sample mixtures</th>
<th>Mean % recovery of analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>COP</td>
<td>10.95</td>
<td>4.85±0.32</td>
<td>90.6-103.4</td>
<td>96.99</td>
</tr>
<tr>
<td></td>
<td>DPH</td>
<td>13.5</td>
<td>9.81±0.80</td>
<td>90.1-106.1</td>
<td>98.07</td>
</tr>
<tr>
<td>B</td>
<td>COP</td>
<td>6.0</td>
<td>4.90±0.57</td>
<td>86.6-109.4</td>
<td>98.00</td>
</tr>
<tr>
<td></td>
<td>DPH</td>
<td>11.0</td>
<td>10.16±0.86</td>
<td>93.0-110.02</td>
<td>101.56</td>
</tr>
<tr>
<td>C</td>
<td>COP</td>
<td>5.7</td>
<td>4.95±0.34</td>
<td>92.2-105.8</td>
<td>98.99</td>
</tr>
<tr>
<td></td>
<td>DPH</td>
<td>14.0</td>
<td>10.05±0.44</td>
<td>96.1-104.9</td>
<td>100.50</td>
</tr>
<tr>
<td>D</td>
<td>COP</td>
<td>10.95</td>
<td>5.12±0.63</td>
<td>89.8-115.0</td>
<td>102.40</td>
</tr>
<tr>
<td></td>
<td>DPH</td>
<td>13.50</td>
<td>10.00±0.29</td>
<td>97.1-102.9</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 2. Zero-order spectra of 0.1 mg/ml diphenhydramine hydrochloride (A) and codeine phosphate (B) standards in 0.1 M HCl showing $\lambda_{\text{max}}$ at 258 nm and 284 nm, respectively.

Simultaneous determination of codeine phosphate and diphenhydramine hydrochloride in combined mixtures. The spectrophotometric results was not significantly different ($p < 0.05$) when compared with those obtained for the official HPLC method BP (2008). The 95% confidence interval between the accuracy and precision (evaluated by the student t-value) between the spectrophotometric method and the BP (2008)
Figure 3. Zero-order derivative spectra of Diphenhydramine hydrochloride and codeine phosphate (0.1 mg/ml) as binary components for Samples A to D.

specification and method for single dosage form for codeine phosphate (95.00 to 105.00%) and diphenhydramine HCl (90.00 to 110.00%) was not statistically different.

The findings in this study indicates that the zero-derivative UV spectrophotometric technique enable the use of simple sample preparation and cost-effective instrumentation with readily available solvent in the laboratory. This technique unlike some techniques in literature (Matsul and French, 2006; Maren et al., 2009) does not require any dye or complexing reagent, special pre-treatment of sample or matrix of actives, thus, making it free from interference by common excipients as confirmed by their recovery study and statistical comparison with the official HPLC method. It is therefore quite reproducible, simple and accurate, which can be used for the routine quality control of pharmaceutical formulations containing the binary components of codeine phosphate and diphenhydramine hydrochloride.

Conclusion

The zero-order derivative UV spectrophotometric technique, developed in this study, has proved to be suitable for the simultaneous determination of codeine phosphate and diphenhydramine hydrochloride in commercial mixtures.

REFERENCES


Hood DJ, Cheung HY (2003). A chromatographic method for rapid and


