Simultaneous HPLC-determination of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol in multi-component combinations

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ABSTRACT

A RP-HPLC method for quantification of analgin (ANA), caffeine (CAF), domperidone (DOM), ergotamine tartarate (ERGTAR) and paracetamol (PAR), singley or admixed in multi-component pharmaceutical preparations, was developed, optimized and validated. The analyzed drug substances could be elegantly separated on a reversed phase column[Nucleosil C18 (10µm, 15cm×4.6mm, i.d.)] isocratically by using a mixture of sodium dihydrogen o-phosphate (0.02M) - methanol (30:70, v/v) as the mobile phase with UV-detection at 240 nm. Significant linearity was observed in the ranges of 54-600µg mL-1 (ANA), 18-180µg mL-1 (CAF), 10-900µg mL-1 (DOM), 1-45µg mL-1 (ERGTAR) and 30-300µg mL-1 (PAR). The challenge of the developed method is its suitability for the successful separation and quantification of each of the named drug substances; either in their laboratory-prepared mixtures and/or in the complex matrices of pharmaceutical preparations containing them in single run. Statistical evaluation of the results was obtained by adopting the proposed method and those of official ones has been undertaken by applying the student t-testing, F-ratio calculation and by one-way ANOVA assessment.

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KEYWORDS

Analgin; Caffeine; Domperidone; Ergotamine; Paracetamol; RP-HPLC.

INTRODUCTION

Analgin and paracetamol are commonly prescribed analgesics, while caffeine is a central nervous stimulant, domperidone is a specific dopamine blocker usually recommended as an antiemetic. Ergotamine is a semi-synthetic dopamine D2-agonist usually prescribed as antimigrainic drug (Figure 1) [2]. Medicines containing different combinations of them are normally intaken for the relief of severe migrain headache [3].

Chromatography is a widely adopted methodology for the analysis and stability investigations of most drugs in pharmaceutical formulations and in quite similar complex matrices. Different chromatographic procedures, such as high-performance liquid chromatography (HPLC) [3-7] and/or thin-layer chromatographic (TLC) fractionation coupled with densitometric scanning [8,9] have been suggested for the determination of
HPLC-determination of analgin, caffeine, domperidone, ergotamine tartarate

Different chromatographic methods, such as HPLC-analysis\cite{10-13}, or gas chromatography (GC)\cite{14} have been suggested for the determination of caffeine and paracetamol separately or combined in some pharmaceutical preparations. Several analytical methods, like HPLC\cite{15-19} and/or coupled chromatography/densitometry\cite{20,21} are described for the quantification of domperidone in various pharmaceutical formulations.

Several HPLC methods\cite{22-25} have been suggested for the determination of ergotamine in some dosage forms.

However, all of the cited methods don’t include a procedure for simultaneous determination of all the named five drug substances in their multi-component mixtures, especially in cases of complex matrices, like dosage formulations. In modern analytical laboratory, there is always a need for significant method for analyzing such multi-component dosage forms.

The main aim of this work was to develop a simple and fast but accurate analytical method for quantifying analgin, caffeine, domperidone, ergotamine tartrate and paracetamol simultaneously in multi-component dosage formulations containing most or even all of them. In a link-frame of cooperation between pharmaceutical industry and universities, such a target could be achieved with affording great time and effort-saving through the complete analysis of all the named substances in two different pharmaceutical preparations (tablets) by adopting the optimized and validated methodology. Satisfactory short complete analysis-time (~8 minutes) could be achieved by following the described experimental conditions.

**EXPERIMENTAL**

**Chemicals and reagents**

HPLC-grade methanol, Sodium dihydrogen orthophosphate and tartaric acid.

**Samples**

**Pure reference samples**

All reference substances were kindly supplied by R&D-unit at ADCO, Cairo-Egypt: Analgin, Zhejiang Haisen Pharm. Co. Ltd., Dongyang city, Zhejiang-China, BNo.:2007-06050, 100.39±0.81% pure, according to the BP-2008 method (volumetry)\cite{2}. Caffeine, Sinochem Ningbo Chem. Co. Ltd., Ningbo City, Zhejiang-China, BNo.:200705177, its purity was 99.25±0.96%, as assayed by the BP-2008 method (titrimetry)\cite{3}. Domperidone, Dr. Reddy’s Pharm. Co., Greenlands, Hyderabad-India, BNo.:Dplm 049D06, purity 100.02±0.79%, as assayed by the BP-2008 method (HPLC)\cite{2}. Ergotamine tartrate, Biesterfeld International
±0.37 %, was assessed by the BP-2008 method (spectrophotometry)\(^2\). Paracetamol, 99.86 \(\pm 0.87 \) % pure, as determined by the method described in the BP-2008 (HPLC)\(^2\).

**Stock standard solutions**

- Standard solutions were stable for at least a week on keeping refrigerated \((\sim 5^\circ C)\).
- Standard stock methanolic solutions of each drug substances;
- Analgin stock standard solution \((1.8\text{mg mL}^{-1})\)
- Caffeine stock standard solution \((0.6\text{mg mL}^{-1})\)
- Domperidone stock standard solution \((3\text{mg mL}^{-1})\)
- Ergotamine tartrate stock standard solution \((0.2\text{mg mL}^{-1})\)
- Paracetamol stock standard solution \((1.5\text{mg mL}^{-1})\) and their mixtures were prepared by careful complete dissolution of accurately weighed aliquots of the substance(s) in calculated volumes of methanol.

**Apparatus and experimental conditions**

Liquid chromatograph consisted of an isocratic pump, a variable wavelength UV-detector, equipped with autosampler injector and integrator (Model 1100 \(\amalg\)).
Series, Agilent USA). Stationary phase: Nucleosil C_{18} analytical column (10μm, 15cm×4.6mm, i.d.), Altech (USA). Mobile phase composed of 20mM NaH_{2}PO_{4} solution and CH_{3}OH (30:70, v/v (pH 5.3±0.2)) was running isocratically at 1.5mL min^{-1}. The mobile phase was filtered through a 0.45-μm millipore membrane and was degassed for about 15 minutes in an ultrasonic bath prior to use. The rate of flow was controlled at 1.5mL min^{-1}, isocratically at ambient temperature (~25°C) with UV-detection at 240nm. The samples were filtered also through a 0.45-μm membrane filter.

Calibration

Aliquot volumes of analgin (1.8mg mL^{-1}), caffeine (0.6mg mL^{-1}), domperidone (3mg mL^{-1}), ergotamine tartarate (0.2mg mL^{-1}) and paracetamol (1.5mg mL^{-1}) stock solutions were accurately transferred separately into a series of 100-mL volumetric flasks, and the content of each was completed to volume with methanol to cover the concentration ranges of 54-600 μg mL^{-1} (ANA), 18-180 μg mL^{-1} (CAF), 10-900 μg mL^{-1} (DOM), 1-45 μg mL^{-1} (ERGTAR) and 30-300 μg mL^{-1} (PAR). The samples were then chromatographed by considering the following chromatographic conditions: Stationary phase; a C_{18}-Nucleosil column (10μm, 15cm×4.6mm, i.d.) from Altech Associates, Inc.(Deefield, Il-USA), mobile phase NaH_{2}PO_{4}-solution (20mM) -methanol (30:70, v/v), filtered and ultrasonicated prior to use. Sample volumes each of 5μL were injected in replicates. To reach good equilibria, the analysis was usually performed not before passing ~50-60mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. The relative peak area values were plotted versus their corresponding concentrations to get the calibration graphs and to compute the corresponding regression equations.

Concentrations of unknown samples of ANA, CAF, DOM, ERGTAR and PAR were determined by using the obtained regression equation.

Analysis of laboratory prepared mixtures

Laboratory prepared mixtures containing different ratios of ANA, CAF, DOM, ERGTAR and PAR were prepared, as detailed in TABLE 2, and the mixtures were chromatographed as under the calibration curves starting from: “5μL were injected...”. The concentration of each component was calculated from its corresponding regression equation.

Analysis of pharmaceutical dosage forms

Twenty tablets were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to one tablet was accurately weighed and transferred into a 100mL volumetric flask. The mixture was shaken with 1mL 1% (w/v) aqueous tartaric acid solution and 50mL of methanol (to achieve complete dissolution of ergotamine tartarate). The solution was subjected to ultrasonic bath for 10 min and the volume was completed with methanol and filtered through filter paper. Further dilution was made to obtain the proper concentrations using methanol as diluting solvent then chromatographed as described under the construction of calibration curves starting from: “5μL were injected...”. The concentration of each component was calculated from its corresponding regression equation.

RESULTS AND DISCUSSION

The literature does not contain any HPLC methods for the simultaneous assay of ANA, CAF, DOM, ERGTAR & PAR in mixtures. In the present work, a simple, accurate, and sensitive HPLC method for the simultaneous determination of them has been developed, validated, and applied for the quantitation of these five drug substances in pharmaceutical dosage forms.

Method optimization

Choice of stationary phase

Different types of stationary phase C_{8} and C_{18} col-
Columns with different dimensions and particles size were tried (Agilent C$_8$ Zorbax, Agilent C$_18$ Zorbax, Agilent C$_8$ Eclipse and Agilent C$_18$ Eclipse columns), to get the best stationary-mobile phase match. It was clearly found that Nucleosil[(ODS), 10μm (15cm×4.6mm, i.d.)] gave the most suitable resolution for quantification of all the named five drug substances.

**Choice of mobile phase**

Different mobile phases at different pH values and varying organic modifiers including acetonitrile and methanol have been tested for optimizing the HPLC-separation. The mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. The optimum mobile phase composition, with a final pH of 5.3 [±0.2], was found to be sodium dihydrogen o-phosphate (20mM) in bidistilled water - methanol, in the ratio of 30:70, by volumes. Flowing at rate of 1.5mL min$^{-1}$ was found to be quite satisfactory for the good resolution and determination of all the studied drug substances, singly and/or admixed. Increasing the ratio of sodium dihydrogen o-phosphate or decreasing the flow rate leads to delay in the elution of all peaks, also decrease in ratio of sodium dihydrogen o-phosphate or increase in flow rate leads to bad resolution between all peaks.

**Choice of detector wavelength**

For determination of the optimum HPLC-UV detector wavelength, the method was repeated using the same chromatographic conditions at different wavelengths (220-300nm), where, the optimum wavelength with ideal sensitivity and low noise was at 240nm and is quite far from the cut-off of water and methanol.

Upon applying the optimum chromatographic conditions, the method was used for the analysis of laboratory prepared mixtures of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol.

### TABLE 2: Analysis of laboratory prepared mixtures of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol by the proposed HPLC method

<table>
<thead>
<tr>
<th>Ratio$^a$</th>
<th>Recovery (%)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td>CAF</td>
</tr>
<tr>
<td>300</td>
<td>25</td>
</tr>
<tr>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>-</td>
<td>15</td>
</tr>
</tbody>
</table>

$^a$Different postulated ratios of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol, respectively.

$^b$Average of 3 experiments

### TABLE 3: Summary of the validation parameters of the proposed HPLC method for the determination of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ANA</th>
<th>CAF</th>
<th>DOM</th>
<th>ERGTAR</th>
<th>PAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.0055</td>
<td>0.017</td>
<td>0.0341</td>
<td>0.0335</td>
<td>0.0066</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.0019</td>
<td>-0.0105</td>
<td>-0.0176</td>
<td>-0.0024</td>
<td>+0.0096</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9999</td>
</tr>
<tr>
<td>Range (μg/mL$^{-1}$)</td>
<td>54-600</td>
<td>18-180</td>
<td>10-900</td>
<td>1-45</td>
<td>30-300</td>
</tr>
<tr>
<td>Accuracy : Mean ±RSD (%)</td>
<td>99.49±0.840</td>
<td>99.86±0.640</td>
<td>99.82±0.212</td>
<td>99.87±0.488</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precision (RSD%)</th>
<th>ANA</th>
<th>CAF</th>
<th>DOM</th>
<th>ERGTAR</th>
<th>PAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability$^c$</td>
<td>0.126-0.071</td>
<td>0.282-0.176</td>
<td>0.524-0.473</td>
<td>0.387-0.192</td>
<td>0.619-0.535</td>
</tr>
<tr>
<td>Intermediate precision$^c$</td>
<td>0.552-0.471</td>
<td>1.473-1.016</td>
<td>1.34-0.982</td>
<td>0.684-0.132</td>
<td>0.853-0.712</td>
</tr>
<tr>
<td>Limit of detection (μg/mL$^{-1}$)</td>
<td>10.20</td>
<td>5.44</td>
<td>1.61</td>
<td>0.207</td>
<td>6.00</td>
</tr>
<tr>
<td>Limit of quantitation (μg/mL$^{-1}$)</td>
<td>30.91</td>
<td>16.47</td>
<td>4.88</td>
<td>0.627</td>
<td>18.18</td>
</tr>
</tbody>
</table>

$^c$The intraday (n=6), average of six concentrations repeated three times within the day.

$^c$The interday (n=6), average of six concentrations repeated three times in three successive days.
dition, well resolved sharp peaks of ANA, CAF, DOM, ERGTAR & PAR, appeared at retention times of ~1.59, 2.39, 3.64, 4.69 and 1.94 minutes in order. Only very little practical deviations from the mean retention times of the resolved drugs were observed, but not in the same days. The total run time for a complete quantification of all the five drug substances was ~8 minutes. Figure 2 shows a typical chromatogram obtained from the analysis of a laboratory prepared mixture of reference ANA, PAR, CAF, DOM & ERGTAR, in order, by using the proposed method. Figures 3 & 4 show typical chromatograms obtained from the analysis of the two commercial multi-component mixtures Amigrain™ and No-migrain® tablets using the proposed method.

**System suitability**

System suitability parameters calculated under the optimized experimental conditions. These five components could be eluted in forms of symmetrical peaks quite away from each other and the retention time values of the separated peaks together with other chromatographic parameters are collected in TABLE 1. The TABLE describes the calculated resolution values (R_S) as well as selectivity factor (α) which ensures complete or 100% separation of the components under investigation. The Tailing factor of each drug peak also revealed linear isotherm peak elution without tailing.

**Method validation**

**Range and linearity**

Linear relationships were obtained between relative peak areas and concentrations for ANA, CAF, DOM, ERGTAR & PAR in concentration range of 54-600μg mL⁻¹, 18-180μg mL⁻¹, 10-900μg mL⁻¹, 1-45μg mL⁻¹ and 30-300μg mL⁻¹, respectively. The regression equations were computed from the relative peak area of each drug substance (peak area of drug to that of external standard 180μg mL⁻¹, 60μg mL⁻¹, 300μg mL⁻¹, 30μg mL⁻¹ and 150μg mL⁻¹ for ANA, CAF, DOM, ERGTAR and PAR in order) versus their corresponding concentrations (TABLE 3).

**Limit of detection and limit of quantification**

For each standard, The limit of detection (LOD) was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be reliably detected, at a signal-to-noise (S/N) ratio. Determination

**TABLE 4 : Comparison between the results of the analysis of the studied drug substances in two tablets formulations by the proposed HPLC-method and the official (BP-2008) methods**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAR</td>
<td>ERGTAR</td>
</tr>
<tr>
<td>Sample-1 tablets</td>
<td>-</td>
<td>104.38±0.607</td>
</tr>
<tr>
<td>Sample-2 tablets</td>
<td>99.30±0.174</td>
<td>101.00±0.575</td>
</tr>
<tr>
<td>SAT¹ (Mean±RSD %)</td>
<td>99.00±0.848</td>
<td>98.92±1.326</td>
</tr>
</tbody>
</table>

¹HPLC-analysis, ²Titrimetry (volumetry), ³UV-Analysis, ⁴Standard addition technique (all results are average of five experiments)

**TABLE 5 : Statistical comparison of the results obtained by the proposed HPLC-method for determination of pure samples of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol with the results of the official (BP-2008) methods**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>The proposed HPLC method</th>
<th>Official method[2]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANA</td>
<td>CAF</td>
</tr>
<tr>
<td>Mean</td>
<td>100.97</td>
<td>99.49</td>
</tr>
<tr>
<td>Concentration range(μg mL⁻¹)</td>
<td>54-600</td>
<td>18-180</td>
</tr>
<tr>
<td>SD</td>
<td>0.743</td>
<td>0.836</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.736</td>
<td>0.840</td>
</tr>
<tr>
<td>Variance</td>
<td>0.552</td>
<td>0.699</td>
</tr>
<tr>
<td>F-value (5.005)⁷ n=6</td>
<td>1.197</td>
<td>1.305</td>
</tr>
<tr>
<td>Student's t-test(2.228)⁷ n = 6</td>
<td>1.153</td>
<td>1.415</td>
</tr>
</tbody>
</table>

⁴Titration (volumetry), ⁵Potentiometric titration, ⁶UV-method, ⁷Figures in parentheses represent the corresponding tabulated values of t and F at p=0.05
of the S/N ratio was performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. An S/N ratio of 3:1 is generally considered acceptable for estimating the detection limit.

Limit of quantitation (LOQ) was determined by establishing the minimum concentration at which the analyte can be reliably quantitated. An S/N ratio of 10:1 is generally considered acceptable for estimating the quantitation limit.

According to ICH\cite{28,29} recommendations the approach based on SD-values of the responses and the corresponding slopes, the detection and quantitation limits were calculated. The theoretical values were assessed practically as they are given in TABLE 2.

Accuracy

To study the accuracy of the proposed method, laboratory prepared mixtures containing various amounts of ANA, CAF, DOM, ERGTAR & PAR were prepared and analyzed by the proposed method. The mean percentage recovery and SD were calculated from the recovery experiment and compared with official methods for the same compounds in similar pharmaceutical preparations. Results are presented in TABLE 2.

Precision

The precision of the proposed method, expressed as RSD\%, was determined by analysis of 3 different concentrations within the linearity range for each ingredient. The intraday precision was assessed from the results of 6 replicate analyses of same concentration on a single day. The interday precision was determined from the same concentration analyzed on 3 consecutive days. The results of intraday and interday precision are illustrated in TABLE 3.

Specificity

For testing the specificity of the method, the percentage recovery of each component was determined in mixture with possible interfering materials, excipients. In application of the proposed methods to pharmaceutical formulation no interference from the tablet’s excipients appeared. Hence the proposed method is able to determine the named drugs selectively in their pharmaceutical preparations. Standard addition technique (SAT) has been also applied to assess the accuracy and specificity of the proposed method, as shown in TABLE 4.

Robustness

The robustness of a method is its ability to remain unaffected by small changes in parameters. Several modified chromatographic conditions, small changes in proportions of different components, by up to ±0.5\% mainly of the organic part of the mobile phase, in addition to the ionic strength of the o-phosphate salt component, flow rate, pH of the mobile phase (5.3±0.2) and different production lot number of Nucleosil C\textsubscript{18} column, were applied which did not affect the good separation of the five components.

Stability

Analyzing commercial samples kept at room temperatures (~22±0.5°C) on the laboratory bench or in the refrigerator (~5°C) for two weeks has been carried out which resulted in RSD\% values within 1.0%.

Statistical analysis\cite{30,31}

Statistical evaluation of the results obtained by applying the proposed method and those of the Official (BP-2008) ones has been undertaken by the student $t$-testing, $F$-ratio calculation and by one-way ANOVA assessment, where it was concluded that there is no statistically significant differences between them (TABLE 5).

CONCLUSION

The proposed HPLC method is simple, and the total run time for the chromatographic run is less than 8 minutes for the 5 components of Amigrain\textsuperscript{TM} tablets No-migrain\textsuperscript{®} tablets. The quantitation of each component was not affected by any of the possible interfering substances included in tablet manufacturing. The method is accurate and precise, as is evident from the results of the recovery study and the low RSD\% values. It can be concluded that the proposed HPLC method has great promise for the routine determination of cited drugs single, combined in laboratory prepared mixtures and in the pharmaceutical preparations.
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