Spectrotdensitometric simultaneous determination of esomeprazole and domperidone in human plasma

Abstract: A simple, rapid, cost-effective, and sensitive TLC-spectrotdensitometric method for simultaneous determination of esomeprazole and domperidone was developed and tested in human plasma. Ethyl acetate: methanol: benzene: acetonitrile (5: 4: 8: 3, v/v/v/v) mobile phase was used for separation on TLC plates detected at 286 nm. The linearity ranges were 5-1200 and 2-600 ng/spot for esomeprazole and domperidone, and limits of detection were 1.73 and 0.59 ng/spot. The effects of four variables affecting Rf were evaluated by fractional factorial design. The benzene volume and saturation time had significant effects.

Keywords: Esomeprazole; domperidone; UV spectrotdensitometry; fractional factorial design; human plasma

1 Introduction

Esomeprazole (ESO) is a benzimidazole used as an anti-ulcer agent and for treatment of reflux esophagitis [1]. Domperidone (DOMP) is a selective dopamine D2 receptor antagonist that increases peristalsis and prevents vomiting [2] (Figure 1). DOMP is usually administered with ESO to prevent ulcers. The analytical techniques used to determine this mixture have been spectrophotometry [3-5] and one TLC method [6].

Our goal was to develop a TLC separation followed by spectrotdensitometry and simultaneous determination in human plasma. Design of Experiment is based on mathematical and graphical representations of combinations of factors. It is used in method optimization and validation; it also saves time and effort in experimental design. The fractional factorial design approach is mainly used when nonlinear response is expected [7-10]. Robustness – the independence of small experimental variations – can be examined, and the method can be revised if the results are not satisfactory [11].

2 Experimental

2.1 Instrumentation

A Camag TLC scanner with Linomat 5 (Switzerland); Merck (Germany) silica gel TLC sheets (F254 plates 20 × 20 cm, 0.25 mm thickness); UV lamp (Marne-la-Vallée cedex, France) and a Hamilton TLC spotting syringe (LKB, Bromma, Sweden) were used. All data analysis was performed using Design-Expert software (trial version 10, Minneapolis, USA).

2.2 Materials and reagents

Solvents were of analytical-reagent grade. Methanol, benzene, ethyl acetate and acetonitrile were purchased from Fisher Scientific, United Kingdom. ESO was kindly supplied by NODCAR, Giza, Egypt and DOMP was kindly supplied by EPICO, 10th Ramadan city, El-Sharquia, Egypt.

3 Standard solutions

Accurately weighed ESO (20 mg) and DOMP (10 mg) were transferred to a 25 mL volumetric flask containing 10 mL methanol and sonicated for five minutes, then filled to the mark with methanol. The ESO and DOMP concentrations...
were 800 and 400 µg mL⁻¹, respectively. Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL were transferred to 10 mL volumetric flasks and filled to the mark with methanol.

2.4 Human plasma solid phase extraction

Five replicates of three concentrations were performed for each drug. Human plasma (1.0 ml) in a centrifuge tube (Eppendorf, Hamburg, Germany) was spiked with standard solution, vortexed for 10 minutes, and mixed with 15 mL acetone. The supernatant was separated and centrifuged at 1000 rpm for 5 minutes. C₁₈ cartridges (Waters, USA) were conditioned with 2.0 mL methanol followed by 5.0 mL ultrapure water. The sample extracts were passed through the cartridge at 0.2 mL/min followed by washing with 2.0 mL/min ultrapure water. The cartridge was hot-air dried and the drugs eluted with 10.0 mL methanol at 0.1 mL/min. The eluate was concentrated to 0.5 mL and vortexed for about ten minutes, then 1 mL acetonitrile was added and centrifuged at 10000 rpm for another 10 minutes. The supernatant was transferred to a clean tube for TLC.

2.5 Chromatographic conditions

The mobile phase was ethyl acetate: methanol: benzene: acetonitrile (5: 4: 8: 3, v/v/v/v). The tank was saturated with the mobile phase for 30 min at room temperature (25± 1ºC) before use. A microliter syringe was used to spot samples as 5 mm bands on silica gel F254 (10 cm × 10 cm) plates. Development was continued until the solvent front reached three fourths of the plate length. The plates were then air-dried for approximately 5 min. A Camag TLC scanner in reflectance-absorbance mode from 250-350 nm was used to measure the chromatograms.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and discussion

3.1 Selection of optimized conditions

3.1.1 System development

After several trials, distinct and well-separated spots were obtained with the ethyl acetate: methanol: benzene: acetonitrile (5: 4: 8:3, v/v/v/v).

3.1.2 Spectra

The UV spectra (Figure 2) were recorded to determine the ESO-DOMP mixture isoabsorptive point (286 nm).

3.2 Method validation

3.2.1 Linearity, LOQ and LOD

Figures 3A-3C show that increasing concentrations increases the peak areas. The correlation coefficient (r) and coefficient of variation (CV) are in Table 1. Limits of detection (LOD) and quantitation (LOQ) were calculated from: LOD or LOQ = ys/S where y=10 for LOQ and 3.3 for LOD, S is the mean slope while s is the intercept standard deviation [12].

3.2.2 Reproducibility

Sample application reproducibility was demonstrated by measurement of the peak area at three concentration levels (Table 2).

3.3 Robustness

Fractional factorial design (FFD) was applied to study the robustness using a four factors half fractional design (2⁴−). The factors were selected based on their effects during
the observed runs: volume of benzene (A), chamber saturation time (B), wavelength (C), and solvent front (D). The deviation of Rf from the original value as their values were changed from the optimum settings are in Table 3. Response surfaces and perturbation plots show the effects on Rf. Perturbation plots reveal the change with all other factors held constant, and slope curvature indicates the sensitivity to the individual factors. The Pareto chart is useful for checking significance, where effects above the Bonferroni Limit are almost certainly significant; effects above the t-value are significant and smaller effects are not.

Small variations in both benzene volume and chamber saturation time had important effects. As can be seen from the response surface and contour plots, an increase in benzene content increased Rf (Figure 4).

Figure 2: Absorption spectra of EZM and DOM showing their isoabsorptive point.

Figure 3: (A) Three dimensional chromatogram of EZM-DOM mixtures. (B) TLC chromatogram showing well separated peaks of EZM and DOM. (C) Calibration curves for EZM and DOM.
ANOVA results are summarized in Table 4. The impacts can be identified by comparison of the factor coefficients. A model p > 0.05 indicates that factors had insignificant effect on Rf, demonstrating a robust method. Adequate precision when S/N > 4 and the known concentration ratio was obtained indicated an adequate signal. The relative standard deviation and adequate precision indicates a good relationship between the experimental data and the fitted models.

4 Analysis of synthetic mixtures

Several synthetic mixtures were analyzed (Table 5). The coefficient of variation was not more than 2.6%.

Table 1: UV-spectrodensitometric method.

<table>
<thead>
<tr>
<th></th>
<th>EZM</th>
<th>DOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>200-1200</td>
<td>100-600</td>
</tr>
<tr>
<td>Regression equation</td>
<td>66.0±2.48X</td>
<td>53.3±6.09X</td>
</tr>
<tr>
<td>Correlation coefficient (r)± s*</td>
<td>0.9994±0.02</td>
<td>0.9989±0.02</td>
</tr>
<tr>
<td>Intercept standard deviation</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Slope standard deviation</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>Intercept % relative standard deviation</td>
<td>1.96</td>
<td>2.06</td>
</tr>
<tr>
<td>Slope % relative standard deviation</td>
<td>2.01</td>
<td>1.64</td>
</tr>
<tr>
<td>LOD (ng/spot)</td>
<td>1.73</td>
<td>0.59</td>
</tr>
<tr>
<td>LOQ (ng/spot)</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

* Average of six replicates.

Table 2: Repeatability and reproducibility.

<table>
<thead>
<tr>
<th>EZM: DOM ratio (ng/spot)</th>
<th>Repeatability % Recovery ± SD*</th>
<th>% RSD</th>
<th>Reproducibility % Recovery ± SD*</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EZM</td>
<td>DOM</td>
<td>EZM</td>
<td>DOM</td>
</tr>
<tr>
<td>300:200</td>
<td>99.9±2.3</td>
<td>2.3</td>
<td>99.7±2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>500:400</td>
<td>102.4±1.8</td>
<td>1.8</td>
<td>103.3±1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>700:600</td>
<td>97.9±1.7</td>
<td>1.7</td>
<td>98.6±2.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* Average of six replicates.

Figure 4: (A) Pareto chart, (B) Perturbation plot, (C) Three-dimensional response surface plot and (D) contour plot showing factor effects interaction of EZM and DOM on Rf.
5 Application to the determination of ESO and DOMP in human plasma

Fresh human plasma from healthy volunteers was frozen at -20°C until use. The drug mixture was added at three concentrations covering the linearity ranges of both drugs (Table 6) then analyzed. Unspiked plasma was the blank (Figure 5A). While good separation and clear identification was obtained in the spiked samples (Figure 5B), neither drug was above the LOD in blanks. Hence, no blank corrections were required.

6 Conclusion

An efficient TLC-spectrodensitometric method employing solid phase extraction optimized by fractional factorial design was developed for determination of esomeprazole and domperidone. Compared to the HPTLC method, this has advantages of cost-effectiveness, sensitivity and a detection wavelength far enough from solvent
interferences. The method showed good precision and sensitivity. It was applied to determination of these drugs in human plasma.

**Conflict of interest:** Authors state no conflict of interest.

**References**


