Simultaneous Determination of Diclofenac Potassium and Drotaverine Hydrochloride in Human Plasma using Reversed-Phase High-Performance Liquid Chromatography

Prasad P. Dahivelkar1,*, Suvarna I. Bhoir2, Sanjay B. Bari1, Sanjay J. Surana1 and Ashok M. Bhagwat2

1Department of Pharmaceutical Analysis, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India – 425 405, and
2Department of Chemistry, S. V. K. M.’s C. B. Patel Research Institute, Vile-Parle, Mumbai, India

Received 31 August 2010; revised 26 May 2011

A simple high-performance liquid chromatographic method with ultraviolet detection is proposed for the estimation of diclofenac potassium and drotaverine hydrochloride in human plasma. Liquid–liquid extraction was carried out with a mixture of dichloromethane–isopropyl alcohol (80:20, v/v). Chromatographic separation of the analytes and internal standard was achieved on an analytical 250 × 4.6 mm i.d. reversed-phase Thermo BDS Hypersil C8 (5 µm particle size) column using a mobile phase of acetonitrile–0.02M ammonium acetate buffer (53:47, v/v) at pH 3.5. The run time was less than 15 min. Column eluate was monitored at 230 nm. The linearity over the concentration ranges of 25–1500 ng/mL and 32–960 ng/mL was obtained for diclofenac potassium and drotaverine hydrochloride, respectively. The limit of quantification was 25 and 32 ng/mL for diclofenac potassium and drotaverine hydrochloride, respectively. Recoveries of diclofenac potassium and drotaverine hydrochloride from plasma were 97.45% and 98.27%, respectively.

Introduction

Diclofenac potassium (DP), [2-(2,6-dichloaroamino)phenyl]acetic acid, is a non-steroidal anti-inflammatory and antipyretic drug. It is usually administered as a potassium (Figure 1) or sodium salt (1–4). It inhibits prostaglandin synthesis by interfering with the action of prostaglandin synthetase (cyclooxygenase) (5, 6). Several types of analytical procedures have been proposed for the estimation of diclofenac in pharmaceutical formulation, including potentiometry (7), fluorimetry (8), gravimetry (9), UV spectrophotometry and partial least squares regression (PLS) (10).

Many high-performance liquid chromatography (HPLC) methods are reported for determination of diclofenac (11–15) in which different columns are used, such as C18, Inertil ODS-3, Zorbax C8 and Macherey–Nagel Nitrile columns; the mobile phases used are, for example: methanol–water–acetic acid (80:20:0.5); 0.1M ammonium acetate buffer–methanol (15.85, v/v); acetonitrile–0.05M disodium hydrogen orthophosphate buffer (60:40, v/v) at pH = 3.5; 0.01M each of sodium acetate and glacial acetic acid buffer–methanol (85:15, i) at pH = 4.6.

Drotaverine hydrochloride (DH), 1-{[3,4-Diethoxy phenyl]methylene}-6,7-diethoxy-1,2,3,4-tetra hydro isoquinolene (Figure 1), is an analogue of papaverine. DH is officially in the Pharmacopoeia of Poland (16). It acts as an antispasmodic agent by inhibiting the phosphodiesterase IV enzyme, specifically to smooth muscle spasms and relieve pain, and is often used to reduce excessive labor pain (17).

Various articles have been published (18–25) indicating the use of the ultraviolet (UV) spectrophotometry technique for estimation of drotaverine in pharmaceuticals. Other reported analytical techniques include computer-aided spectrophotometry (26), potentiometry (27) and polarography (28). Some reversed-phase (RP)-HPLC methods (29–37) have been reported for estimation of drotaverine in pharmaceuticals and biological samples.

A thorough literature survey has revealed HPLC methods (31–33) using perchlorate ions, which feature the shortcomings of longer system equilibration (30–45 min) and reduced column efficiency over time. The proposed method avoids the use of perchlorate in the mobile phase and is suitable for pharmacokinetic study. Studies reported by Mezei et al. (34), Girgis et al. (35) and Lalla et al. (36) have estimated drotaverine with a lower limit of quantitation (LLOQ) of 50 ng/mL, whereas the present method has an LLOQ of 32 ng/mL.

A combination of diclofenac and drotaverine renders antispasmodic activity and is administered to avert excessive labor pain. The co-administration calls for a bioanalytical method to separate and quantify these drugs in the presence of each other. Few studies have reported the estimation of these drugs in aqueous media. A literature survey also confirmed the absence of any reported RP-HPLC method for the simultaneous estimation of DP and DH in human plasma, which justifies the need for the present work.

A literature survey reveals no information about the short-term and long-term plasma stability of diclofenac and drotaverine during a simultaneous determination by HPLC. This article presents results for short-term and long-term plasma degradation studies of the two drugs. Other authors have reported a separate method for the estimation of drotaverine hydrochloride and mefenamic acid (37). The present study provides the outcome of stability studies for the combination of DP and DH. This method has been successfully applied in estimation of both drugs in human plasma (in vivo study) and can be applied for therapeutic drug monitoring.

The liquid–liquid extraction (LLE) method opted here involves only two steps, ensuring a simple and fast extraction with better recovery data than solid-phase extraction (SPE). This method confirms sensitivity and presents advantages like enhanced chromatographic column life, economic viability,
better accuracy and a shorter run time by abiding by the U.S. Food and Drug Administration (FDA) bioanalytical method validation guidance (38).

**Experimental**

**Chemicals and reagents**

DP, DH and mefenamic acid (internal standard; IS) were supplied by Glen-Mark Pharmaceuticals (Nasik, India). All solvents were of HPLC grade (E-Merck, Mumbai, India). HPLC-grade water was obtained by double distillation in glass, through an RO water purification system (Canpex, Mumbai, India). Water was filtered through 0.22 µm filters (Millipore, Mumbai, India). Blood samples were collected from healthy volunteers and separated plasma was stored at 20°C.

**Preparation of stock and working standard solution**

The stock solutions of DP, DH and IS were made up in methanol to concentrations of 980, 921.14 and 50 µg/mL. High quality control (HQC), medium quality control (MQC), low quality control (LQC) and linearity range dilutions were obtained by serially diluting the stock solution with mobile phase. The amount of IS, mefenamic acid, was 2,500 ng in each sample tube.

**Instruments and chromatographic conditions**

The HPLC method was performed using a Jasco HPLC system (Jasco HPLC Systems, Mumbai, India) comprising of an Intelligent UV-Vis detector (UV-1575), a 3-line degasser (DG-1580), an Intelligent HPLC pump (PU-1580) and Borwin Chromatograph software (Mumbai, India). Chromatograms were run at ambient temperature on a steel C8 Thermo BDS Hypersil (250 mm x 4.6 mm, 5 µm) column. The mobile phase, containing a mixture of acetonitrile and 0.02M ammonium acetate buffer (53:47, v/v) at pH = 3.5, was pumped at a flow rate of 1 mL/min with UV detection at 230 nm. Organic solvent system used for LLE was dichloromethane–isopropyl alcohol (80:20, v/v).

**Choice of IS**

Mefenamic acid was selected as an IS because of its ready availability and good stability under working conditions. The IS was well resolved from DP and DH. The tailing factor for the mefenamic acid peak was 1.34.

**Procedure for sample extraction**

One milliliter of blank plasma was placed in 15-mL conical centrifuge glass tubes (J-sil Borosil, Mumbai, India). For the preparation of calibration curves, internal controls (high, medium and low) made of 25 µL each of DP and DH serial dilutions prepared from stock solutions were added to conical centrifuge glass tubes, separately. The mixture of plasma and stock solution was vigorously vortex-mixed for 1 min. From the stock solution, 50 µL of IS was added and vortex-mixed for 30 s. One milliliter ammonium acetate buffer solution was added and the mixture was again vortex-mixed for 1 min. The second step of LLE involved the addition of 5 mL organic solvent system (extraction solvent). The mixture was vigorously vortex-mixed for 2 min, then centrifuged for 1,509 g for 10 min. The organic layer (4 mL) was separated. A nitrogen sample evaporator (Takahe Instruments, Mumbai, India) at 30°C was used to evaporate the organic solvents. The residue was reconstituted with 200 µL mobile phase and an aliquot (20 µL) was injected into the HPLC column.

**Figure 1.** Chemical structures of the analytes.
Blood samples were collected from healthy volunteers and plasma was also obtained. *In vivo* plasma levels of DP and DH were determined by following the same extraction procedure. All studies were performed under the instructions of the ethical committee of the S.V.K.M’s C.B. Patel Research Institute (Vile-Parle, Mumbai, India).

**Methodology for validation**

A thorough and complete method validation of DP and DH in human plasma was performed by abiding by the U.S. FDA guidelines for bioanalytical method validation (38). The method was validated for selectivity, linearity, limit of detection (LOD) and limit of quantification (LOQ), precision and accuracy, recovery, dilution integrity, matrix effect, robustness, ruggedness and stability.

To test selectivity, ten different lots of blank plasma (with K3 EDTA as anticoagulant) were processed by the same procedure for sample extraction and analyzed to determine the extent to which endogenous plasma components interfere at the retention times of analytes and IS. In conforming to selectivity criteria, from each of these 10 different lots, two replicates each of 950 µL were spiked with 50 µL of acetonitrile–0.02M ammonium acetate buffer (53:47, v/v). In the first set, the blank plasma, without analyte and IS, was directly injected after extraction, while the other set was spiked only with IS before extraction (for total of 20 samples). Furthermore, one system suitability sample (SSS) at the second level of calibration standard (CS-2) concentration and two replicates of LLOQ concentration, i.e., calibration standard-1 (CS-1) were prepared by spiking blank plasma (5% of total volume of plasma) with combined working aqueous standards of DP and DH. The blank plasma sample was used for spiking of SSS and LLOQ was chosen from one of these 10 lots of plasma.

The linearity of the method was evaluated by analyzing standard plots associated with an eight-point standard calibration curve. Five linearity curves containing eight nonzero concentrations were analyzed. Each of the eight concentrations was prepared by adding 50 µL of IS and 25 µL of each drug, and increasing amounts of DP and DH, separately, to 1 mL of drug-free plasma and extracting the samples as described previously. The final concentrations obtained were 25, 50, 150, 300, 600, 900, 1,200 and 1,500 ng/mL for DP and 32, 64, 160, 320, 480, 640, 800 and 960 ng/mL for DH, respectively. Recovery was evaluated by comparing the peak area of extracted samples (spiked before extraction) to the peak area of unextracted samples (QC working solutions spiked in extracted plasma).

An experiment for the dilution integrity was performed with an aim to validate the dilution test to be carried out on higher analyte concentrations, i.e., more than the upper limit of quantification (ULOQ), which may be encountered during subject sample analysis. The dilution integrity experiment was carried out at five times the ULOQ concentration (i.e., 7,500 ng/mL for DP and 4,800 ng/mL for DH, respectively, and also at the HQC level for both analytes). Six replicate samples each of 1/10 of 5 × ULOQ and 1/10 of HQC were prepared and their concentrations were calculated by applying the dilution factor of 10 against the freshly prepared calibration curve for DP and DH.

The effect of matrix on analyte quantitation with respect to consistency in signal (suppression/enhancement) was checked in six different lots of K3 EDTA plasma. Four replicates, each at LQC and HQC levels, were prepared from these lots of plasma (a total of 48 QC samples) and checked for the accuracy in terms of percent bias in all QC samples. The specificity experiment was conducted for DP, DH and IS by comparing peak areas at their respective retention times.

Robustness testing is vital per FDA guidelines for bioanalytical method development. The parameters selected for robustness study have to reflect potential changes that may occur during the validation process. The reliability of the analytical method is tested when small variations occur in method parameters. The robustness of the method was determined, after analysis of six replicates, based on the use of two analytical C8 columns, with small variations in the proportion of constituents of mobile phase and small changes in the flow rate of the mobile phase. The results were expressed as a function of the CV. An influence of variation in the room temperature was excluded because the temperature of the laboratory was monitored daily and kept at 25 ± 2 °C.

Ruggedness evaluates the precision between repeatability and reproducibility. The analyst should establish the effect of the random events on the precision of the analytical procedure. Ruggedness is assessed by precision when multiple
analyzers perform the same defined assay using multiple columns on multiple days in the laboratory. The considered variables in the ruggedness study included analysts (1 and 2), equipment (column 1 and 2) and days (1 and 2).

The stability of compounds in human plasma was evaluated under different conditions, simulating the same conditions that occurred during study sample analysis. Stock solution stability was performed by comparing the area response of the stability sample of the analytes and IS with the area response of the sample prepared from fresh stock solutions. Bench top stability (BTS), room temperature stability, refrigerated stability of extracted sample (RSS) and freeze-thaw stability were performed at LQC, MQC and HQC levels using six replicates at each level. The long-term stability study was carried out with plasma blank sample spiked with DP and DH, which were stored at −80°C for 3 months and then analyzed periodically for 1 month against a standard curve prepared on the analysis day.

Results and Discussion

Method optimization

The present bioanalytical method was developed and validated for assaying DP and DH in therapeutic concentration ranges for the analysis of routine samples. It was necessary to develop a simple and accurate method for the simultaneous extraction of DP and DH from human plasma because they have different physicochemical properties (3, 16).

Sample extraction

DP has a pKa of 3.8 and mefenamic acid (IS) has a pKa of 4.2. The pKa value for DH is 6.3. Both analytes have different pKa values, and hence, quantitative extraction of the drugs was difficult. Extraction procedures like protein precipitation (PP) and SPE exhibit significantly high noise levels, so LLE was tried and found to work. LLE was tried with different solvent systems, i.e., diethyl ether, DCM, IPA and combinations of solvents like chloroform–IPA–n-heptane in a proportion of 60:14:26, v/v/v; or DCM–IPA–tetrahydrofuran in a proportion of 60:20:20, v/v/v; however, the recoveries were not encouraging in any of these solvents. A combination of DCM–IPA in a proportion of 80:20, v/v was tried and found to give better recoveries. The addition of ammonium acetate buffer helped to give consistent and reproducible response for the analytes and IS in DCM–IPA (80:20, v/v). The validation results and subject sample analysis support this extraction methodology, and hence, the method was accepted in the present study.

High performance liquid chromatography

Because DP and DH have different pKa values and polarities, it was difficult to set chromatographic conditions that produced sharp peak shapes and adequate response. This included mobile phase selection, pH of buffer solution, flow rate, column type and injection volume. Different volume ratios of methanol–water and acetonitrile–water combinations were tried as mobile phase, along with ammonium acetate, ammonium formate buffers and sodium dihydrogen orthophosphate buffers of varying strengths on C18 Kromasil (250 × 4.6 mm, 5 μm) and C8 Thermo BDS Hypersil (250 × 4.6 mm, 5 μm) columns. It was observed that acetonitrile–0.02M ammonium acetate buffer (53:47, v/v) at a pH = 3.5 as a mobile phase, with a flow rate of 1 mL/min, was the most appropriate for faster elution and better efficiency and peak shape. An aqueous part (47%) was adequate to retain the relatively polar compound DP on the C8 column. The C8 column, compared to C18, has provided better separation without compromising key chromatographic separation parameters like resolution and number of theoretical plates (N). The use of the C8 column helped in the separation and elution of all three compounds, with a total run time of 12 min.

Method validation

Selectivity

The aim of performing a selectivity check with 10 different types of plasma batches (plasma of healthy Indian subjects with K3 EDTA as an anticoagulant) was to ensure the authenticity of the results for study sample analysis. Figure 2 illustrates representative chromatograms of drug-free human plasma, blank plasma spiked with drugs and IS and plasma of a volunteer administered with the drug combination. Figure 2A confirms blank plasma peaks at 3.67, 3.84, 4.29, 5.55, 6.26, 7.78 and 8.91 min. No interfering peaks were observed in blank plasma samples. Retention times were 7.08, 8.60 and 10.07 min, respectively, for DP, DH and IS (Figure 2B). Figure 2C demonstrates the absence of interfering endogenous compounds in plasma from a volunteer consuming the drug combination.

Linearity

The calibration curves for DP and DH were linear from 25–1500 ng/mL for DP and 32–960 ng/mL for DH. Typical calibration plots for plasma extracts had good correlation coefficients (Table I).

LOD and LOQ

The LLOD of DP and DH (three times the baseline noise) were 9 and 11 ng/mL, respectively. The LOQ, defined as the lowest concentration that could be measured with accuracy and precision, i.e., within ±20% of the actual value, was 25 and 32 ng/mL for DP and DH, respectively.

Accuracy and precision

Method performance was evaluated as intra-assay accuracy and precision, determined by five replicate analyses for DP and DH at three concentration levels, i.e., LQC, MQC and HQC, each on the same analytical run. Inter-assay precision and accuracy were calculated after repeated analysis in three different analytical runs. The results are listed in Table II. These results show the repeatability of the method, including both sample processing and chromatographic measurement. The CV (%) is a ratio of standard deviation to mean in percent. Small deviations from perfect accuracy were observed (i.e., −2.78% at most), whereas the CV (%) was 5.77 at most. Inter-assay CV determined from experiments performed on three days (n =
Recovery

Recovery was determined by dividing the peak area obtained from analysis of DP and DH added to plasma by that observed for the same amount of standard added to mobile phase and 18) was 4.61 at most (Table II). These statistics indicate good precision.

Recovery

Recovery was determined by dividing the peak area obtained from analysis of DP and DH added to plasma by that observed for the same amount of standard added to mobile phase and

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Obtained for Linearity, LOD and LOQ of the HPLC Method</td>
</tr>
<tr>
<td>Statistical parameter</td>
</tr>
<tr>
<td>Concentration range (ng/mL)</td>
</tr>
<tr>
<td>Regression equation</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
</tr>
<tr>
<td>LOD ng/mL</td>
</tr>
<tr>
<td>LOQ ng/mL</td>
</tr>
</tbody>
</table>
injected into the chromatograph. Recoveries of DP and DH from plasma were 97.45 and 98.27%, respectively.

**Dilution integrity**

A dilution integrity (DI) test was performed that established the accuracy and precision results within the acceptable criteria of ±15% for each dilution. The DI test for diclofenac at a concentration level of dilution five times higher than ULOQ concentration offered a recovery of 96.3% (RSD = 2.4%). The DI test at the HQC concentration level resulted in a recovery of 97.2% with a RSD of 1.9%. A similar DI test for drotaverine at concentration level five times higher than the ULOQ provided a recovery of 96.7% (RSD = 2.7%). The HQC level concentration DI test for DH presented a recovery of 98.3% with an RSD of 1.6%.

**Matrix effect**

The matrix effect is due to coelution of some components present in biological samples. These components can dramatically decrease or increase the analyte response to affect the sensitivity, accuracy and precision of the method. The assessment of matrix effect constitutes an important and integral part of validation for quantitative RP-HPLC methods supporting pharmacokinetics studies. Assessment of the matrix effect was done to observe the effects of different lots of plasma on the back-calculated values of QC's nominal concentrations. The results were well within the acceptable range.

**Robustness**

The robustness of the method for DP and DH measurements was determined by involving small variations in the chromatographic system, using two analytical C8 columns of the same make with different serial numbers, 1 to 2% acetonitrile in the mobile phase and 0.05 mL/min changes on the flow rate of the mobile phase. Three plasma concentrations, LQC, MQC and HQC, were measured for each assay. The two C8 analytical columns were compared and the results, as expressed by CV were −0.35 to 1.6% for DP and −0.47 to 3.1% for DH. The mobile phase composition showed a marked impact on separation. A slight increase in acetonitrile percentage (to 60%) caused a general reduction of retention time, with no major concerns over separation and no overlapping of peaks. Table III presents CVs for the estimation of diclofenac and drotaverine after changing the mobile phase composition. Finally, small changes on the flow rate of the mobile phase were tested and CVs were calculated (Table III).

**Ruggedness**

The MQC concentration levels were selected for both drugs. Each experiment was repeated three times to evaluate the experimental error variance. No considered factor was found significant for the proposed method. The relative standard deviations (RSDs) found was acceptable, indicating an acceptable precision of the analytical procedure.

**Method application**

**Stability**

The stability of DH and DP in plasma was determined under various conditions according to the procedure described previously. A short-term stability test performed at room temperature showed that three quality control samples were stable for 12 h (Table IV), with average mean recoveries of 99.5, 100.6 and 98.9% for DP and 100.3, 99.8 and 100.9% for DH, respectively. The long-term stability results indicated that DP and DH samples were stable for 3 months (Table IV), with average recoveries of 96.5, 98 and 99.1% for DP and 96.1, 99.1 and 98.8% for DH, respectively. No significant change of DP and DH concentration in plasma was detected after exposing samples to three freeze-thaw cycles, and mean recovery was found to be 99.2 ± 0.6% for DP and 100.5 ± 0.5% for DH, respectively. Finally, the stability was also determined in the processed sample ready for injection. Results showed that three QC samples were stable at least for 8 h with CVs below 5%.

---

**Table II**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diclofenac potassium (ng/mL)</th>
<th>Drotaverine hydrochloride (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual value (ng/mL)</td>
<td>75</td>
<td>450</td>
</tr>
<tr>
<td>Intra-assay precision and accuracy</td>
<td>Mean concentration</td>
<td>75.7</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>4.37</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>5.77</td>
</tr>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diclofenac potassium (% CV)</th>
<th>Drotaverine hydrochloride (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two C8 analytical columns</td>
<td>−0.35</td>
<td>−2.60</td>
</tr>
<tr>
<td>Mobile phase composition</td>
<td>2.23</td>
<td>4.61</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.57</td>
<td>3.35</td>
</tr>
</tbody>
</table>

**Table IV**

<table>
<thead>
<tr>
<th>Parameter / condition</th>
<th>Diclofenac potassium (ng/mL)</th>
<th>Drotaverine hydrochloride (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical amount</td>
<td>75</td>
<td>450</td>
</tr>
<tr>
<td>Short-term stability (8 h)</td>
<td>Mean initial concentration</td>
<td>75.2</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>Mean final concentration</td>
<td>74.8</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>−0.53</td>
</tr>
<tr>
<td></td>
<td>Long-term stability at −80°C (90 days)</td>
<td>Mean initial concentration</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>Mean final concentration</td>
<td>71.5</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>−3.51</td>
</tr>
</tbody>
</table>
Bioavailability and bioequivalence study

Bioanalytical methods are available for individual estimation of both drugs. The present bioanalytical method for simultaneous estimation is proven to be an economical, relatively accurate, simple and less time-consuming alternative.

Conclusion

In summary, an isocratic RP-HPLC method based on LLE with UV detection is used for the determination of diclofenac potassium and drotaverine hydrochloride in human plasma. No interference of endogenous compounds was found in the examined samples, which confirms the good selectivity of the method. The proposed simultaneous extraction procedure is considerably efficient, rapid and sensitive for routine analysis. Employing acetate buffer instead of perchlorate in the mobile phase is advantageous because of the consequent deterioration of endogenous compounds was found in the examined samples, which confirms the good selectivity of the method. Finally, the HPLC method presented here is rapid, sensitive, specific and particularly useful for processing multiple samples for pharmacokinetic studies.

Acknowledgments

Authors are thankful to Glen-Mark Pharmaceuticals, Nasik, India for availability of free drug samples.

References


33. Akesiripong, S., Janwittayanuchit, W., Ratanajamitara, C., Juthong, S.; Comparative bioavailability study of drotaverine hydrochloride; *Hua Chiew University Journal*, (1999); 3(5): 7–12.


