**ABSTRACT**

Objective: A simple, sensitive and rapid performance liquid chromatography/positive ion electrospray tandem mass spectrometry method was developed and validated for quantification of propafenone (PPF) and its two major metabolite 5-hydroxy propafenone (5-OHP) and N-depropyl propafenone (NDP) in human plasma.

Methods: Liquid-liquid extraction (LLE) with ethyl acetate was used of extraction of plasma samples. The analytes were separated using an isocratic mixture of 0.1% formic acid/acetonitrile (20:80 v/v) on a reversed-phase column Hypurity Advance C18 50 x 2.1 mm, 5µ and analysed by mass spectrometry in the multiple reaction monitoring mode using the respective [M+H] ions. The m/z was 342.20/116.10 for propafenone, m/z 299.80/74.10 for N-depropyl propafenone and m/z 358.30/98.10 for 5-hydroxy propafenone along with m/z 409.2/238.0 for Amlodipine as internal standard respectively.

Results: The method had a short chromatographic run time of 1.5 min. The method exhibited a linear dynamic range over 5.11 to 1000.73 ng/ml for propafenone, 0.51 to 100.6 ng/ml for N-depropyl propafenone and 5.11 to 1001.64 ng/ml for 5-hydroxy propafenone respectively, in human plasma.

Conclusion: The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetics, bioavailability and bioequivalence studies.

Keywords: Stability, N-depropyl propafenone, 5-hydroxy propafenone, Amlodipine, Validation, LC-MS

**INTRODUCTION**

Propafenone hydrochloride (PPF) is an antiarrhythmic drug with a phenyl propanolamine nucleus that is common to various beta blocking agents. PPF is known to be effective in the treatment of supraventricular and ventricular arrhythmias [1]. It is known to act by blocking the fast inward sodium impulse in the cardiac muscle and all other excitable tissues. PPF also possesses some beta blocking action as well as a weak calcium channel blocking effect [2]. PPF is primarily metabolized by hydroxylation of the ring to form 5-hydroxypropafenone [3] (5-OHP) whereas a small amount of N-depropylpropafenone (NDP) is also formed. The excretion of PPF is mainly in the form of glucuronide and sulfate conjugates of PPF, 5-OHP and NDP [4]. While the primary metabolite 5-OHP exerts pharmacological activity equivalent to the parent drug, a little is known about the action of NDP [5].

Several methods have previously been reported for the determination of PPF and its metabolites in human plasma using liquid-liquid extraction and solid phase extraction techniques [6-9]. Most of these methods either have very long run times or tedious extraction processes involved for analysis of the sample mixture. A few methods including the one developed by Lipig Pan et al. [10] and Sheshagiri Rao et al. [1] are known to have smaller run times of 6 and 4 min respectively. This paper describes a very simple liquid chromatography-mass spectrometry (LC-MS) method for estimation of PPF, 5-OHP and NDP in human plasma using liquid-liquid extraction technique and a very short acquisition time using amlodipine besylate as the internal standard (IS).

**MATERIALS AND METHODS**

**Chemicals and reagents**

All the solvents used were of HPLC grade or higher and all the other chemicals were of analytical grade or higher. PPF, NDP and 5-OHP were supplied by Splendid Lab, India. HPLC grade methanol and acetonitrile were purchased from J.T. Baker; formic acid AR (85%) was procured from Finar fine chemicals. Ethyl acetate was purchased from Rankem. Blank human plasma was procured from the blood bank Sai laxmi lab, Hyd. Amlodipine besylate was obtained from Splendid Lab, India. Milli Q-water was used throughout the study.

**LC-MS/MS instrumentation and conditions**

The HPLC System (Shimadzu) was equipped with autosampler SIL-HTC, solvent delivery mode LC-10ADvp and column oven CTO-10ASvp. The chromatography was performed on a Thermo Hypurity Advance C18 (50 x 2.1 mm, 5µ) column at 40 °C. The isocratic mobile phase was acetonitrile: 0.1% formic acid (80:20 v/v) which was pumped at 0.3 ml/min. Injection volume was 5 µl and total run time was 1.5 minute. Mass spectrometric detection was performed on AB SCIEX API 3200 with positive ionization mode. Mass transition [M+H] ions, m/z was 342.20/116.10 for PPF, m/z 299.80/74.10 for NDP and m/z 358.30/98.10 for 5-OHP along with m/z 409.2/238.0 for Amlodipine.

**Preparation of standard solutions**

An accurately weighed quantity of 11.07 mg, 10 mg and 11.02 mg of propafenone, N-depropyl propafenone and 5-hydroxyl propafenone respectively was transferred in 10 ml volumetric flask and volume was made up to 10 ml with methanol. All stock solutions of 1 mg/ml were prepared and stored between 2-8 °C until the time of use.

**Mixture of stock solution**

An accurately measured quantity of 1 ml of stock solution of PPF, NDP and 5-OHP was transferred in 25 ml of volumetric flask and diluted up to mark with diluents. The solution was stored in refrigerator between 2-8 °C.

**Preparation of stock solution**

The IS of concentration 100 µg/ml was prepared by dissolving an accurately weighed quantity of 13.14 mg of amlodipine besylate in
100 ml of methanol. Accurately measured 5 ml of IS stock solution was transferred to a 100 ml volumetric flask and the volume was made up with diluent.

**Spiking in pooled plasma**

The plasma lots were thawed and pooled at room temperature and a volume of 9.5 ml of the screened pooled plasma was transferred to 10 ml of volumetric flask and spiked with 0.5 ml of spiking solution to obtained all non-zero standards and quality control samples (Low, Medium and High) and vortexed to ensure proper mixing of analytes. Non-zero standards were 5.11 to 1000.73 ng/ml for PPF, 0.51 to 100.06 ng/ml for NDP and 5.11 to 100.64 ng/ml for 5-OHP.

**Preparation and extraction of drug from plasma samples**

Aliquot (0.475 ml) of spiked plasma of non-zero standard and quality control samples were taken in RIA vials and 25 µl of IS stock solution (5.0 µg/ml) was added to it and vortexed for 15 seconds. Approximately 3.0 ml of extraction solvent was added to the spiked plasma samples and was vortexed for 2 min. The mixture was centrifuged at 3500 rpm for 5 min at 4 °C and the plasma layer was flash-frozen. The organic layer was transferred into a pre-labeled tube. The sample was evaporated to dryness at 40 °C under the stream of nitrogen the dried extract was reconstituted with 500 µl of mobile phase and vortexed for 15 seconds and transferred into pre-labeled vials. 5 µl of the analyte was injected into chromatographic system. For preparation of blank samples 5% dihuent was added to the pooled plasma and the extraction was done as per the process reported for drug sample.

**Bioanalytical method validation**

All standard stock solution of PPF, NDP and 5-OHP of 1 mg/ml were prepared separately up to stock dilution. A mixture of all three analytes was finally prepared. Stock solution of IS was prepared in methanol. Spiking solution for calibration and quality control were prepared by appropriate dilution in methanol: water (90:10 v/v). Spiking solution (0.5 ml) was added to drug free human plasma (9.5 ml) as a bulk to obtained concentration levels of 5.11, 11.35, 75.66, 168.12, 336.25, 560.41, 800.59 and 1000.73 ng/ml for propafenone, 0.51, 1.13, 7.56, 16.81, 33.62, 56.03, 80.05 and 100.06 ng/ml for N-depropyl propafenone and 5.11, 11.36, 75.72, 168.28, 336.55, 560.92, 801.32 and 1001.64 ng/ml for 5-Hydroxy propafenone. Quality control samples (Low, medium, high) were also prepared at concentration 15.36, 336.29 and 800.59 ng/ml for propafenone, 1.53, 33.28 and 80.05 ng/ml for N-depropyl propafenone, 15.36, 336.44 and 801.32 ng/ml for 5-hydroxy propafenone. The spiked samples were stored in freezer at below-20 °C until analysis.

Calibration curve was constructed from blank sample and eight non-zero standards covering the stated range including LLOQ. The calibration curve was generated using the analyte to IS peak area ratio by weighted (1/X) least squares linear regression on consecutive days. The acceptance criterion was a correlation coefficient (r) of 0.99 or higher, and each back calculated standard concentration must be within 15% deviation except at the LLOQ which the maximum acceptable deviation was set at 20%.

The method developed for determination of PPF, NDP and 5-OHP in human plasma was validated for assay specificity, sensitivity, matrix effect, recovery, linearity, precision, accuracy and stability in accordance with the USFDA guidelines.

**Specificity**

The chromatogram obtained from six different lots, apart from lipemic and hemolytic blank human plasma was identified and retrieved in order to analyze the potential interference of endogenous substances at the peak region. The specificity of the method was evaluated by comparing chromatograms of blank plasma, blank plasma spiked with PPF, NDP, 5-OHP and IS.

**Matrix effect**

Blank plasma samples were extracted and spiked with PPF, NDP, and 5-OHP at three low concentration levels in six different blank matrices to evaluate the matrix effects of plasma. The corresponding peak areas were compared with those of the standards solutions, and peak area ratio was defined as the matrix effect.

**Recovery**

The extraction recovery of PPF, NDP, 5-OHP and IS was evaluated by comparing the bioanalytical results for the extracted QC samples with solutions equivalent to 100% recovery of low, medium and high QCs. Six replicates for each QC level were performed with the established extraction procedure.

**Calibration curve**

The calibration curve was prepared by analyzing spiked calibration samples at eight different concentrations. Every calibration standard was injected in five replicates. The linearity of calibration curve was assessed by linear regression. The low limit of quantification (LLOQ) was determined by analyzing five replicates of spiked samples.

**Accuracy and precision**

The within run accuracy and precision batches were assessed by analyzing six replicates of LLOQ, Low QC, medium QC, high QC samples and all batches were meeting acceptance criteria.

**Stability studies**

The stability of PPF, NDP, 5-OHP and IS in human plasma was evaluated under different temperature and condition as short term stock solution stability at room temperature and refrigerator (2-8 °C). Long term stock working solution stability, bench top stability, freeze thaw stability, auto-sampler stability and dry extract stability (20 °C±5).

**RESULTS**

Representative chromatogram and calibration for PPF, NDP, and 5-OHP are illustrated in fig. 1(A to C), 2(A to C), 3(A to D) and 4A, 4B and 4C respectively. The retention times of the PPF, NDP, 5-OHP and IS were approximately 0.63, 0.61, 0.60 and 0.62 min. The overall chromatography run time was 1.5 min.
Fig. 1B: Representative chromatogram of blank human plasma sample of N-Depropyl propafenone

Fig. 1C: Representative chromatogram of blank human plasma sample of 5-Hydroxy propafenone

Fig. 2: Representative chromatogram of the lower limit of quantitation sample of PPF (A), NDP (B), and 5-OHP (C)
Specificity

Blank human plasma samples were subjected through the extraction procedure and chromatographed to determine the extent to which endogenous human plasma components may contribute to chromatographic interference with the PPF, NDP, 5-OHP and IS. No significant interference was observed in six different lots of human plasma samples.

Matrix effect

One calibration curve along with 18 low QC samples (three each from six different lots of human plasma) was processed and analyzed with freshly processed calibration samples in a single run. The % nominal value of PPF, NDP, and 5-OHP were found to be 97.75, 103.85 and 104.43 respectively. The results are presented in table 1.

Table 1: Matrix effect

|        | PPF Conc (ng/ml) | mean±SD (%) | NDP Conc (ng/ml) | mean±SD (%) | OHP Conc (ng/ml) | mean±SD (%) |
|--------|------------------|-------------|------------------|-------------|------------------|-------------|
| LQC    | 15.36            | 97.75±0.79  | 1.53             | 103.85±0.11 | 15.36            | 104.43±1.05 |

All data are represented as mean±SD; n=6

Recovery

Recovery of analyte and metabolites was evaluated by comparing mean analyte response of six extracted samples of low, medium and high quality control samples to mean analyte response of six replicates injection of un-extracted quality control samples.

The mean recovery for LQC, MQC and HQC of PPF are 40.51, 47.32 and 52.62, for NDP are 82.01, 99.54 and 102.33 and for 5-OHP are 42.19, 47.64 and 54.81 respectively. The mean recovery for internal standard is 47.90

Calibration curve

Calibration curves of PPF, NDP, and 5-OHP were found to be consistently accurate and precise over the range 5.11 to 1000.73 ng/ml, 0.51 to 100.06 ng/ml and range 5.11 to 1001.64 ng/ml respectively. The regression coefficient (r) was greater than or equal to 0.99. Back-calculations were made from the calibration curves to determine PPF, NDP, and 5-OHP concentrations of each calibration standard and a typical calibration curve of PPF, NDP, and 5-OHP are presented in fig. 4A, 4B and 4C.

Accuracy and precision

The between-run accuracy and precision evaluation were assessed by the repeated analysis of human plasma samples containing different concentrations of PPF, NDP, and 5-OHP on separate occasions. A single run consisted of a calibration curve, 6 replicates of low, medium and high quality control samples. The between-run % coefficient of variation and between-run percentage of nominal value of PPF, NDP, and 5-OHP are presented in table 3.

Table 2: Extraction recovery

| QC level | Propafenone (% recovery) | N-depopyl propafenone (% recovery) | 5-OH propafenone (% recovery) |
|----------|--------------------------|----------------------------------|-------------------------------|
| LQC      | 40.51                    | 82.01                            | 42.19                         |
| MQC      | 47.32                    | 99.54                            | 47.64                         |
| HQC      | 52.62                    | 102.33                           | 54.81                         |
| Mean     | 46.82                    | 94.63                            | 48.21                         |
| SD       | 6.07                     | 11.02                            | 6.33                          |
| % CV     | 12.97                    | 11.64                            | 13.13                         |
Replicate concentrations of PPF, NDP, and 5-OHP in human plasma were analyzed for within-run accuracy and precision evaluations. The run consisted of a calibration curve plus a total of 30 spiked samples, 6 replicates each of the lower limit of quality control (LLOQ), upper limit of quality control (ULOQ), and low, medium and high quality control samples.

The within-run % coefficient of variation of PPF ranged from 2.50 to 6.11 while the within-run percentage of nominal value of PPF ranged from 95.69 to 99.71.

Similarly the within-run % coefficient of variation and the percentage of nominal value of NDP ranged from 1.64 to 6.39 and 89.30 to 103.92 respectively.

The within-run % coefficient of variation of 5-OHP ranged from 2.16 to 6.57 whereas the percentage of nominal value of 5-OHP ranged from 93.35 to 109.83.

### Stability

#### Short term stock solution stability at room temperature

One solution each of PPF, NDP, and 5-OHP at working calibration standard at ULOQ level and IS solution at working internal standard level were prepared in diluent from stock solution. Solutions were kept on bench as such at room temperature. After approximately 26 h, fresh solution each of PPF, NDP, and 5-OHP at ULOQ level and internal standard at working standard level, were prepared in the

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**Table 3: Between run accuracy and precision of plasma samples**

| Propafenone | N-Depropyl propafenone | 5-OH propafenone |
|---|---|---|
| Conc (ng/ml) | mean±SD | % CV | % Accuracy | Conc (ng/ml) | mean±SD | % CV | % Accuracy | Conc (ng/ml) | mean±SD | % CV | % Accuracy |
| 15.36 | 14.95±0.795 | 5.32 | 97.33 | 1.53 | 1.61±0.111 | 6.89 | 105.23 | 1.53 | 1.61±0.111 | 15.36 | 16.09±1.047 | 6.51 | 104.75 |
| 336.29 | 314.59±11.389 | 3.62 | 93.55 | 33.28 | 32.74±1.901 | 5.81 | 98.38 | 33.28 | 32.74±1.901 | 336.44 | 319.44±14.212 | 4.45 | 94.95 |
| 800.59 | 753.45±39.035 | 5.18 | 94.11 | 80.05 | 78.52±3.632 | 4.63 | 98.09 | 801.32 | 795.50±25.702 | 3.23 | 99.27 |

All data are represented as mean±SD; n=6
diluent. Two vials were prepared (each from bench top and freshly prepared) by spiking 50 µl of ULOQ, 50 µl of IS in 900 µl of diluent. Six replicate injections from each vial were given and the area response was used to determine % change over time.

PPF, NDP, 5-OHP and IS are found to be stable in diluent on bench at room temperature for approximately 26 h. The % change was -1.35, -0.19 and -0.60 for PPF, NDP, and 5-OHP respectively whereas for IS it was found to be -0.24.

Table 4: Within-run % CV and % nominal for PPF, NDP and OHP

| Analyte                        | Level | mean±SD   | % CV | % nominal |
|--------------------------------|-------|-----------|------|-----------|
| Propafenone                    | LLOQ  | 4.89±0.242| 4.96 | 95.69     |
|                               | LQC   | 15.32±0.936| 6.11 | 99.71     |
|                               | MQC   | 324.48±8.117| 2.5  | 96.49     |
|                               | HQC   | 786.26±43.46| 5.53 | 98.21     |
| N-Depropyl Propafenone         | LLOQ  | 0.53±0.031 | 5.84 | 103.92    |
|                               | LQC   | 1.47±0.094 | 6.39 | 95.75     |
|                               | MQC   | 29.72±0.487| 1.64 | 93.35     |
|                               | HQC   | 75.10±4.644| 6.18 | 93.82     |
| 5-OH Propafenone               | LLOQ  | 4.77±0.219 | 4.58 | 93.35     |
|                               | LQC   | 16.87±0.548| 3.25 | 109.83    |
|                               | MQC   | 318.68±20.94| 6.57 | 94.72     |
|                               | HQC   | 809.96±17.481| 2.16 | 101.08    |

All data are represented as mean±SD; n=6

**Short term stock solution stability at refrigerator (2-8 °C)**

Stability samples were prepared as for room temperature stability and the solutions were kept into refrigerator as such. After approximately 48 h, fresh solution each of PPF, NDP, and 5-OHP at working calibration level ULOQ level and IS at working IS level were prepared in diluent. Two vials were prepared (each from refrigerator and freshly prepared) by spiking 50 µl of ULOQ, 50 µl of internal standard in 900 µl of diluent. Six replicates from each vial were injected and the area response was used to determine % change over time.

PPF, NDP, 5-OHP and IS were found to be stable in diluent respectively at refrigerator at 2-8 °C for approximately 48 h. The % change for PPF, NDP, and 5-OHP was found to be –0.19, 6.82 and 2.78 respectively whereas for IS the % change was 4.35.

**Bench top stability**

Six samples each of low and high QC (stability samples) were kept on bench at room temperature for approximately 22 h. Stability samples were processed and analyzed (six samples each of low and high QC). Concentrations were calculated to determine % change over time.

Table 5: Bench top stability

| Analyte                        | LQC (% change) | HQC (% change) |
|--------------------------------|----------------|----------------|
| Propafenone                    | -4.29          | 6.74           |
| N-Depropyl Propafenone         | -3.72          | -7.1           |
| 5-OH Propafenone               | -2.3           | 5.08           |

**Freeze thaw stability At–20±5 °C**

Eighteen samples each of low and high QC were retrieved from –20±5 °C after 24 h of storage of samples. After thawing, the stability samples were restored at least 12 h and again the same samples were retrieved and kept on bench at room temperature for thaw. The samples were restored and after at least 12 h again retrieve and thawed. Six stability samples (after three cycles) and six comparison samples at each level (low QC and high QC) were processed and analyzed along with freshly processed quality control samples. Concentrations were calculated to determine % change over time.

Table 6: Freeze thaw stability At–20±5 °C

| Analyte                        | LQC (% change) | HQC (% change) |
|--------------------------------|----------------|----------------|
| Propafenone                    | -3.13          | 3.01           |
| N-Depropyl Propafenone         | 1.39           | -5.86          |
| 5-OH Propafenone               | -1.67          | 0.27           |

**Table 7: Autosampler stability**

| Analyte                        | LQC (% change) | HQC (% change) |
|--------------------------------|----------------|----------------|
| Propafenone                    | 3.06           | 6.02           |
| N-Depropyl Propafenone         | 2.88           | -3.54          |
| 5-OH Propafenone               | 4.35           | 3.8            |
Autosampler stability
Six samples (stability samples) each of low and high QC were processed and kept in auto sampler (at 5±2 °C) for approximately 80 h. The stability samples were analyzed along with freshly processed calibration and comparison samples (six samples each of low and high QC). Concentrations were calculated to determine % change over time.

Dry extract stability (-20±5 °C)
Six samples (stability samples) each of low and high QC was processed and kept in deep freezer (at-20±5 °C) for approximately 81 h. The stability samples were analyzed along with freshly processed calibration and comparison samples (six samples each of low and high QC). Concentrations were calculated to determine % change over time.

Table 8: Dry extract stability (-20±5 °C)

| Analyte               | LQC (% change) | HQC (% change) |
|-----------------------|----------------|----------------|
| Propafenone           | 1.35           | 7.26           |
| N-Depropyl Propafenone| 1.80           | -2.6           |
| 5-OH Propafenone      | 1.56           | 3.76           |

DISCUSSION

Selection of IS
A stable analyte has to be used as IS in order to negate the sample matrix effects. It was difficult to find a compound that could ideally mirror the analyte to serve as a suitable IS. The chosen IS should be able to match the chromatographic properties, recovery and ionization properties of the analyte. Finally amiodipine, a readily available compound, was selected as the IS in positive ion mode. Its chromatographic behavior and extraction efficiency were similar to PPF, NDP and OHP, stable in plasma and reproducible in the LC/MS/MS system. Additionally, it caused no interferences to the analytes under study.

Sample pretreatment
An ideal sample pre-treatment method should be able to remove the interferences from the biological matrix and it must also be reproducible with high recovery in minimum number of steps. Protein precipitation (PPT), liquid-liquid extraction (LLE) and solid-phase extraction (SPE) were tested to obtain a simple and excellent plasma preparation procedure. PPT was easy to dilute the sample, but failed to sufficiently remove endogenous interference. SPE had too steps which contributed to delay in extraction and is expensive too. LLE was carried out with different extraction solvents, including ethyl acetate and diethyl ether, and evaluated for extraction recoveries and matrix effect. LLE was found to be the best suited method as it was able to produce clean chromatograms with sufficient efficiency and specificity. LLE was also able to minimize the ion suppression and matrix effects in LC-MS/MS as well as cost effective hence Ethyl acetate was adopted as the extraction solvent.

Liquid chromatography
The liquid chromatographic conditions, especially the composition of mobile phase, was optimized through several trials to achieve good resolution and symmetric peak shapes for analyte and IS as well as short run time. Various solvent mixtures were tested for different run times in order to obtain the optimal solvent system that would be able to produce the best resolution, sensitivity and peak shape. It was found a mixture of 0.1% formic acid/acetonitrile (20:80 v/v) as an isocratic mobile phase system could achieve this purpose and was finally adopted as the mobile phase. High proportion of organic solvent eluted the PPF, NDP, 5-OHP and amiodipine at retention time of 0.63, 0.61 0.60 and 0.62 min respectively. A flow rate of 0.3 ml/min produced a good peak shape in short run time and hence was used as the mobile phase for eluting PPF and its metabolites.

Mass spectrometry
MS parameters were adjusted for PPF, NDP, OHP and amiodipine in positive ionization mode. The solutions containing the analytes and IS were injected directly into the mass spectrometer and under these conditions the analytes resulted in [M+H] + peaks. Pharmacokinetic application requires highly selective assay with high sample throughput capacity. Quantification of drugs in biological matrices by LC-MS/MS is becoming more common due to the improved sensitivity and selectivity of this technique [12]. Hence to achieve the desired LLOQ Level, LC-MS/MS detection was chosen.

CONCLUSION
A robust LC-MS/MS method was developed and validated for the quantification of propafenone and its two major metabolites in human plasma. The developed method offers an advantage of being rapid and simple with short run time. The simple liquid-liquid extraction technique utilized in the method makes it suitable for the analysis of large sample batches in routine analysis, without any loss in instrument performance.

CONFLICTS OF INTERESTS
All authors have none to declare.

REFERENCES
1. Reimold SC, Maisel WH, Antman EM. Propafenone for treatment to supraventricular tachycardia and atrial fibrillation: a meta-analysis. Am J Cardiol 1998;82:66-71.
2. Dolly C. Therapeutic drugs. 2nd ed. Edinburgh (UK): Churchill living stone; 1999.
3. Kates RE, Yee YG, Winkle RA. Metabolite cumulation during chronic propafenone dosing in arrhythmia. Clin Pharmacol Ther 1985;37:610-4.
4. Hege HG, Hollmann M, Kaumeier S, Lietz H. The metabolic fate of 2H-labeled propafenone in man. Eur J Drug Metab Pharmacokinet 1984;9:41-5.
5. Valkessel C, Delgado C, Tamargo J. Electrophysiological effects of 5-hydroxy propafenone on guinea pig ventricular muscle fiber. J Cardiovasc Pharmacol 1987;10:523-9.
6. Zhong D, Chen X. Enantioselective determination of propafenone and its metabolites in human plasma by liquid chromatography-mass spectrometry. J Chromatogr B 1999;721:67-75.
7. Afshar M, Rouini M. A rapid HPLC assay for the simultaneous determination of propafenone and its major metabolites in human serum. Anal Sci 2004;20:1307-11.
8. Hofmann U, Pezia M, Heinkele G, Dilger K, Kroemer HK, Eichelbaum M. Determination of propafenone and its phase I and II metabolites in plasma and urine by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr B 2000;748:13-23.
9. Kubalcz P, Bransteterova E. Determination of propafenone and its main metabolite 5-hydroxyprefenone in human serum with direct injection into a column-switching chromatographic system. J Chromatogr B 1999;726:211-8.
10. L Pan, Y Qian, Cheng M, Gu P, He Y, Xu L, et al. Pharmacokinetics of propafenone hydrochloride sustained-release capsules in male beagle dogs. Acta Pharm Sin B 2015;5:74-8.
11. Sujan Kumar DP, Palavan C, Sheshagiri Rao JVLN. A rapid and sensitive LC-MS/MS assay for the determination of...
propafenone and its active metabolite 5-hydroxy propafenone in human plasma using SPE precipitation technology. Pharm Lett 2015;7:122-8.

12. Sharma D, Mittal R, Gupta A, Singh K, Nair A. Quantitative bioanalysis by LC-MS/MS: a review. J Pharm Biomed Sci 2010;7:1-9.

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