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DETERMINATION OF PROPAFENONE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

A rapid and specific HPLC method has been developed and validated for the determination of propafenone, an antiarrhythmic agent. Separation was developed on a reverse-phase C18 column (250 mm x 4.6 mm, i.d. 5m) using an acetonitrile-phosphate buffer at a flow rate of 1 ml/min and UV detection of 210 nm. The calibration curves were linear ($r^2 > 0.999$) in the concentration range of 10-100 g/ml. The overall intra- and inter-day variations (RSDs) of the analyte were less than 2% with the recovery ranged within 98102%.

Introduction

Propafenone hydrochloride (PPF) is chemically 2'-[2-Hydroxy-3-(propylamino)-propoxy]-3-Phenyl propiophenone hydrochloride (Fig. 1). Its a class IC antiarrhythmic agent with slight -adrenergic-antagonist properties, which is effective in the treatment of supraventricular and ventricular arrhythmias.1 It is able

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to depress intracardiac conduction velocity as a consequence of its binding to the open state of cardiac Na channels.2 PPF is administered as racemic and the enantiomers have different pharmacological activities and kinetics of disposition.3 Survey of literature reveals that the drug is determined by using GC-MS,4,5 titration,6-8 spectrophotometry,9 HPLC,10-12 and LC-MS13. Regarded to HPLC method, the authors used complex mobile phase14 or gradient mode10. In this study our target to develop a simple HPLC method to determine propafenone and contribute in pharmacopeia monograph instead of current method as titration in British pharmacopeia or United State pharmacopeia.

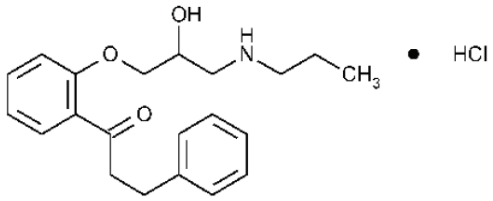

Fig.1 Chemical structure of propafenone

This paper describes a simple, rapid and specific HPLC method with UV detection for the analysis of propafenone. The developed method was successfully applied to the determination of real samples. To our knowledge, this is the first method for the analysis of PPF based on LC methods.

2. Material and Methods

2.1 Materials

Propafenone tablet was purchased in Korea. Propafenone standard was from USP standard (USA). HPLC grade acetonitrile and methanol were from Duksan Pure Chemicals Co. (Ansan, Korea). Other reagent solutions were of analytical grade.

Apparatus and chromatographic condition

The analytical chromatography was performed on a Thermo Scientific Spectra system equipped with P 1000 pump and UV 2000 detector and 20 μl-injection loop. Chromatographic data were acquired and processed by Chromquest 5.0 software.l

The liquid chromatograph was equipped with a 210 nm detector and a 4.6 mm x 250 mm column (ODS C18). The mobile phase consisted of 0.01M monobasic potassium phosphate (KH_2PO_4) buffer at pH 3.0 (using phosphoric acid) and acetonitrile (57: 43) at flow rate of 1 ml/min.

2.3. Sample preparation

Standard stock solution: Dissolve an accurately weighed quantity of USP propafenone hydrochloride in methanol to obtain a solution having a known concentration of about 500 μg per mL and stored at 4^oC. Working solutions were prepared daily from these stock solutions by dilution with buffer.

Sample preparation: Twenty tablets were weighed and ground to a fine powder. An equivalent of the powder corresponding to 100 mg of PPF was weighed and dissolved in a beaker by 100 ml of methanol. This solution was degased and diluted to 500 ml in a volumetric flask (solution A). Solution used for method development: dilute 25 ml solution A into a volumetric flask of 100 ml. This solution was then filtered through filter paper with 0.45 μm before injected.

Calibration curves A series solution containing appropriate concentrations of standard was used for the construction of calibration curves. At least six concentrations of the solution were analyzed in duplicates, and then the calibration curves were constructed by plotting the peak area versus the concentration of analyte.

Precision and accuracy Intra- and inter-day variations were chosen to determine the precision of the developed assay. For intra-day variability test, three concentration of standard were analyzed for six replicates within one day, while for inter-day variability test, three concentration of standard were examined in duplicates for consecutive three days. Variations were expressed by RSD.

Accuracy was determined by recovery test which was performed by adding known amounts of the standard at low (80% of the known amount), medium (same as the known amount) and high (120% of the known amount) levels. Three replicates were performed for the test.

3. Results and discussion

3.1. System suitability

System suitability was tested by performing six replicate injections and determining peak areas, theoretical plate number (N), and symmetry factor (As) for the analyte of interest. The relative standard deviations (RSDs) of these properties were used as indicators of system suitability. The mean value of N for the compound was 11.443, and the As value was 2.0. The RSD of the peak area was 0.80%. All the results showed that the proposed method met the requirement..

Table 1. System suitability parameters of propafenone

Parameter	t_R	Peak area	Theoretical Plate	k '	As
1	5.527	2735983	11352	1.76	1.82
2	5.538	2693918	10867	1.77	2.08
3	5.527	2735225	12158	1.76	2.13
4	5.532	2700552	11103	1.77	2.18
5	5.528	2711205	11635	1.76	1.97
6	5.533	2744243	11543	1.77	2.01
Mean	5.531	2720188	11443	1.77	2.03
RSD%	**0.1**	**0.8**	**3.9**	**0.1**	**6.3**

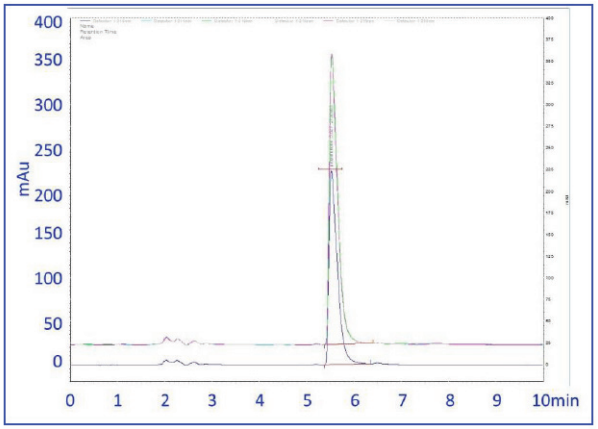

Fig 2. Representative HPLC chromatogram of six times injection of propafenone standard

3.2. Method validation

Specificity The selectivity was evaluated by comparing the retention time of standard reference compound with that of the peaks obtained by analyzing sample.

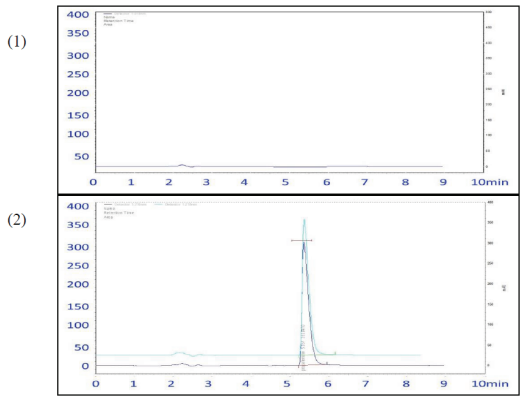

Fig 3. Representative HPLC chromatograms of propafenone at 210 nm, (1) dissolving solvent (methanol), (2) standards and sample of propafenone

The HPLC method was able to discriminate propafenone of pharmaceutical sample from the other constituents of the tablet. There was no interference with the peaks of propafenone in sample (shown in Fig. 3).

Linearity The calibration curves showed that there was a linear dependence of the peak area on the concentration of PPF over the range of 10100 μg/ml in the mobile phase. The equation used to describe the linear relationship between the peak area and the corresponding concentration was y = ax+b. The correlation coefficient (r2) was 0.999. The results of regression analysis were reported in Table 2.

Table 2. Regression curve data of propafenone

Parameter	Data of propafenone
Linearity range (µg/ml)	10 - 100
Slope	67,701.780
Intercept	48,010.981
r^2	0.999

Regression curve data for eight calibration points is y = ax + b, where y is peak area of analytes, x is concentration, a is slope, b is intercept, and r^2 is

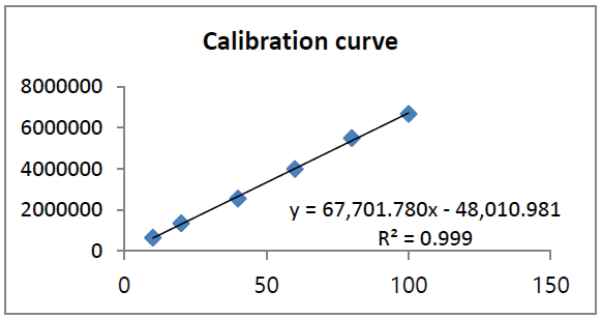

Fig. 4. Calibration curves of propafenone in the range of 10 -100 μg/ml

the squared correlation coefficient.

Precision-Accuracy The results of precision were reported in Table 3. The overall intra- and inter-day variations (RSDs) of the analyte were less than 2% with the recovery ranged within 98102%. The developed method had good accuracy with overall recovery of 98102% with RSD less than 2.0% for the analyte. The recovery test of the method indicated that the treatment of the samples did not result in loss of compound.

Table 3. Intra- and inter-day precision; accuracy for the assay of propafenone

	Concentration (µg/ml)	RSD %	Recovery %
Intra day	40	1.50	99.88
	50	0.50	102.37
	60	1.80	101.32
Inter-day	40	0.01	98.30
	50	0.01	102.0
	60	0.01	98.10
Accuracy	40	0.40	99.20
	50	1.70	100.10
	60	2.00	101.30

Robustness Robustness of the method was determined by making small, deliberate changes in flow rate, pH buffer in mobile phase. Flow rate (1ml/min) was changed by ±0.05mL/min, pH of buffer was changed ±0.2. Their Their dependence on suitability parameter such as retention time and peak area were evaluated. % RSD was ≤ 2 and they were within the limit required for HPLC analysis.

Table 4. Robustness data: retention time and peak areas of propafenone

Parameter	tR		Peak area	
	Mean	RSD (%)	Mean	RSD (%)
		Flow rate (ml/min)		
0.95	5.59	0.04	3,111,125	2.0
1.05	5.06	0.17	3,221,153	1.8
		pH Buffer		
2.8	5.14	0.12	2,703,298	2.0
3.2	5.45	0.11	2,645,608	1.2

3.3. Propafenone determination

Separately inject equal volumes (about 20 μL of the standard preparation and the assay preparation into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculated the quantity, percentage of propafenone hydrochloride ($C_{21}H_{27}NO_3HCl$) in sample taken by the formula:

$$\frac{4Cr_U100}{r_SW}$$

In which C is the concentration, in μg per mL, of USP propafenone hydrochloride RS in the standard solution; r_U and r_S are the propafenone peak area responses obtained from the assay preparation and the standard preparation, respectively; W is the weight, in mg, of the portion of propafenone hydrochloride taken.

Table 5: Result for propafenone determination

	Standard (µg/ml)	Sample (µg/ml)	Sample weigh (mg)	Content (%)
1	50	49.8	200	100.3
2	50	49.6	200	100.7
3	50	50.6	200	98.9
4	50	49.4	200	101.2
5	50	49.3	200	101.5
6	50	50.0	200	99.9
Mean	50	49.8	200	**100.4**

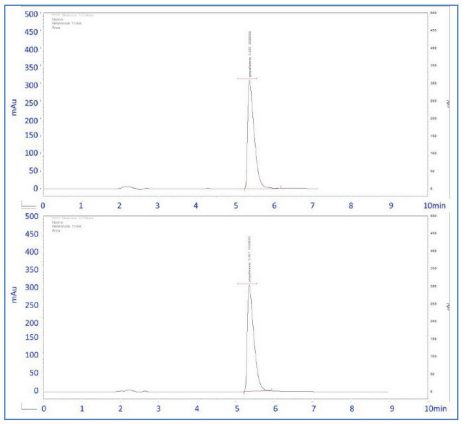

Fig. 5: Typical chromatograph of propafenone assay; mobile phase consisting of 10 mM phosphate buffer (pH 3.0) and MeCN with ratio 57:43 (v/v), Flow rate: 1 mL/min; 210 nm. (1) standard solution 50 μg/mL, (2) sample solution.

Conclusion

The method presented here describes a simple, specific and reproducible assay for the determination of propafenone. The basic characteristics of the HPLC fulfill general validation criteria and thus the method can be used in routine analysis.

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