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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Paracetamol and Tramadol Hydrochloride

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Abstract

A simple and rapid RP-HPLC method has been developed for the simultaneous estimation of paracetamol and tramadol hydrochloride. The chromatographic analysis was carried out on an Enable C18 column (250×4.6 mm i.d., 5µm) at ambient room temperature. The mobile phase used was a 60:40 (v/v) mixture of 10 mM potassium dihydrogen orthophosphate buffer at pH 3: Acetonitrile. Mobile phase flow rate was maintained at 1 ml/min with a run time of 5 minutes and eluents were monitored at 215 nm. The retention times of paracetamol and tramadol hydrochloride were 3.1 seconds and 3.4 seconds respectively. The peaks were sharp, symmetrical and adequately resolved with sufficient theoretical plate count indicating the system suitability. The calibration curves were found to be linear over the concentration ranges 20 µg/mL to 100 µg/mL. The limit of detection of paracetamol and tramadol hydrochloride was 6.19 µg/mL and 5.56 µg/mL respectively. The method validation was carried out and the parameters observed were within acceptable limits.

Keywords: Paracetamol, Tramadol hydrochloride, RP-HPLC, Method validation.

Introduction

Paracetamol (PCM) is a non-opioid analgesic-antipyretic drug that is quite safe at therapeutic doses. The beneficial action is attributed to inhibition of prostaglandin synthesis in the central nervous system. PCM is well absorbed orally, metabolized in liver and excreted in urine. The half-life is 2 to 3 hours. In overdose, a toxic metabolite (N-acetyl benzoquinoneimine) accumulates which results in hepatocellular damage. N-acetyl cysteine is an effective treatment in many cases of toxicity. PCM is chemically N-acetyl-p-aminophenol ($C_8H_9NO_2$) with a molecular weight of 151 and pKa of 9.5. The structural formula is shown in Fig. 1.

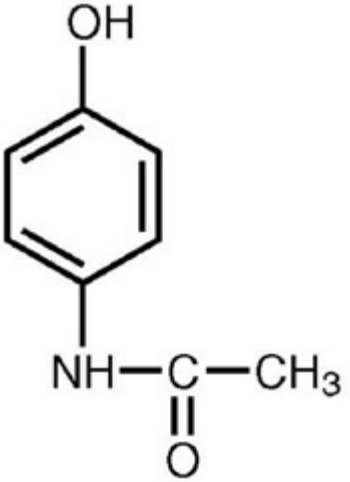

Fig. 1: Structure of Paracetamol

Tramadol hydrochloride (TH) is the salt of tramadol which is a centrally acting opioid analgesic. In addition to its weak agonistic action on µ opioid receptor, it also inhibits the uptake of norepinephrine and serotonin. The O-demethylated metabolite is a more potent analgesic than its parent drug. The elimination half-life of tramadol is 6 hours and that of its active metabolite is 7.5 hours. TH can be administered orally or parenterally for the treatment of moderate to moderately severe pain, and is well tolerated with minimal abuse liability. The most important adverse effect is seizures which happen on overdose and not reversed by naloxone. The chemical name of TH is 2-(dimethylamino methyl)-1-(3-methoxyphenyl) cyclohexanol hydrochloride. Its molecular formula is $C_{16}H_{25}NO_2$ with a molecular weight of 299.8 and has a pKa of 9.41. Structural formula is given in Fig. 2.

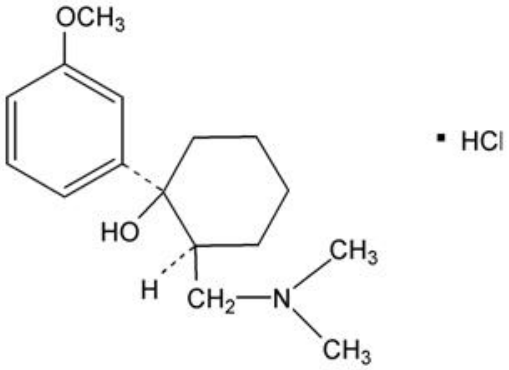

Fig. 2: Structure of Tramadol hydrochloride

Combination of PCM with an opioid analgesic like TH improves its efficacy in moderate pain. This synergistic action is evident by the widespread use of this combination with over 100 commercial formulations. There is need for an analytical method which can simultaneously estimate PCM and TH.

Literature review reveals that simultaneous determination of PCM and TH content in tablets can be achieved by spectrophotometry,[1-7] high performance thin layer chromatography,[8,9] and reverse phase high performance liquid chromatography.[10-21] Among the different analytical equipments and techniques, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) stands out due to its resolution, specificity, accuracy, precision and cost effectiveness. The mobile phase used in the HPLC methods mentioned above is a mixture of either methanol or acetonitrile along with buffer. The important disadvantages of using methanol are the requirement of Poison license to purchase it, higher noise at lower ultraviolet wavelengths (lesser than 250 nm), and higher column pressures. The advantages of using acetonitrile include greater sensitivity for analysis at shorter wavelengths, greater elution strength, and less ghost peaking during gradient technique. The methods mentioned above which utilize acetonitrile have some drawbacks like usage of higher pH i.e. above 7 which can lead to decrease in column life, and usage of phosphate buffer beyond buffering range. In another method, a C8 column was used which is less versatile than the C18 column. Another advantage of the present method is that it avoids the use of triethylamine which can alter the column in a way that is not easily reversible. The present study describes the development and validation of an optimal isocratic RP-HPLC method for simultaneous quantitative estimation of PCM and TH.

Materials and Methods

Chemicals: Pure standards of PCM and TH were purchased from Sigma-Aldrich India. HPLC grade acetonitrile, water, and orthophosphoric acid were purchased from Fischer scientific. Potassium dihydrogen orthophosphate and other chemicals used were analytical grade.

Instrumentation and chromatographic conditions: The HPLC system consisted of a Shimadzu LC 20AD isocratic pump with SPD M20A PDA detector. Chromatographic separation was carried out on an Enable C18 column (250×4.6 mm i.d., 5µm particle size) and a manual injector with a fixed loop of 20 µL volume. The column was equilibrated with mobile phase consisting of 10 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3 with orthophosphoric acid): acetonitrile (60:40 v/v). Mobile phase flow rate was maintained at 1 ml/min with a run time of 5 minutes and eluents were monitored at 215 nm. The mobile phase was vacuum filtered using 0.45 micron nylon filter and degassed using a sonicator for 30 minutes. The standard solution was filtered using 0.2 micron nylon filter before injection. Electronic balance with 0.1 mg sensitivity (Shimadzu, Japan) was used. The pH of buffer solution was measured and adjusted with the help of Eutech Cyberscan pH meter. LC solutions software was used for data acquisition and processing.

Preparation of standard solution: The standard solution was prepared by dissolving 100 mg of PCM and 100 mg of TH in 100 ml buffer and sonicating for 10 min to yield a solution containing 1 mg/mL of PCM and 1 mg/mL of TH. From the filtered solution, serial dilutions were made so as to contain 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL of the drugs.

Calibration curve: The dilutions of standard solution containing 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL of the drugs were injected three times each. The area under curve (AUC) data was obtained from the LC solutions software Peak table. The average AUC of three injections was used for constructing the calibration curve and regression equation.

Method validation

The method developed was validated as per ICH guidelines[22] in terms of linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, robustness and system suitability. Linearity was determined by calculating the correlation coefficient from the calibration curve of each drug. Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equations: $LOD = 3.3\times\sigma/S$, $LOQ = 10\times\sigma/S$; where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve. Precision, expressed as percentage relative standard deviation (%RSD), of the method was verified by performing multiple estimations of the same concentration of both drugs on the same day (for intra-day precision) and on two different days for inter-day precision). Robustness of the method was assessed by making small variations in the method and judging its impact on retention time. The changes made were to the pH of buffer solution (± 0.1 pH) and organic content of mobile phase (± 2%). System suitability was evaluated based on retention time, tailing factor, number of theoretical plates, and resolution with respect to previous peak.

Results and Discussion

Isocratic elution with a mobile phase consisting of 10 mM potassium dihydrogen orthophosphate buffer (pH adjusted to 3 with orthophosphoric acid): acetonitrile (60:40 v/v) at a flow rate of 1 ml/min and monitored at 215 nm resulted in optimal resolution and minimum peak tailing. The retention times of PCM and TH were 3.1 seconds and 3.4 seconds respectively. (Fig. 3, 4)

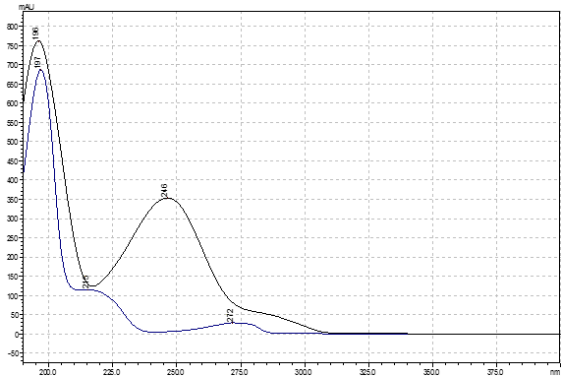

Fig. 3: Overlain spectra of paracetamol and tramadol hydrochloride

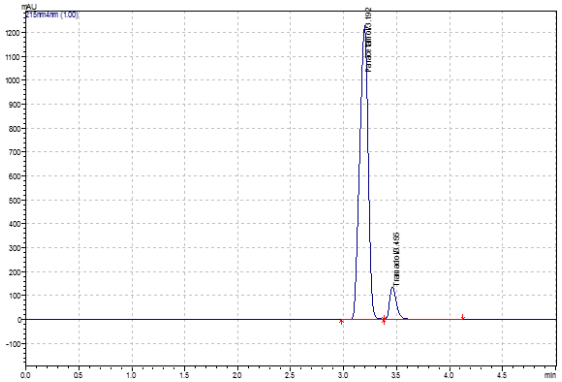

Fig. 4: Chromatograph of paracetamol and tramadol hydrochloride

Linearity and Range

The concentrations of the drugs injected were plotted against the area under curve and the calibration curves (Fig. 5) were drawn. The correlation coefficient indicates the linearity of the plot in a range of 20 µg/mL to 100 µg/mL. The linearity of the method was excellent as evidenced by the correlation coefficient of 0.999 for both drugs. The regression equation and other parameters obtained from the plot are mentioned in Table 1.

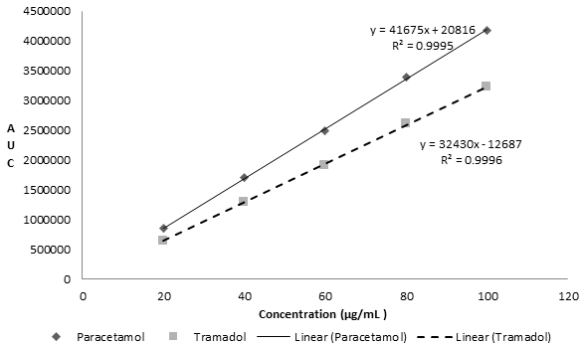

Fig. 5: Calibration curve of paracetamol and tramadol hydrochloride

Table 1: Linear regression analysis data

Parameter	PCM	TH
Range (µg/mL)	20 - 100	20 - 100
Regression equation	y = 41675x + 20816	y = 32430x - 12687
Slope	41675	32430
Intercept	20816	12687
Correlation coefficient	0.999	0.999
LOD (µg/mL)	6.19	5.56
LOQ (µg/mL)	18.77	16.8

Precision: The precision of the method was determined by calculating the intra-day and inter-day variability. For intra-day precision, six injections of a fixed concentration (100 µg/mL) of the drugs were carried out and the variation in AUC expressed as %RSD. For inter-day or intermediate precision, a fixed concentration (100 µg/mL) of the drugs was injected three times each on two separate days and the variation in AUC expressed as %RSD. The intra-day and inter-day precision studies showed that the variation in AUC was well within the acceptable limit of 2% RSD.

Table 2: Intra-day and Inter-day Precision

Drug	Concentration (µg/mL)	Intra-day (%RSD)	Inter-day(% RSD)
PCM	100	0.86	1.13
TH	100	0.79	1.02

Robustness: Small variations in the pH of mobile phase or organic content of mobile phase did not significantly affect the retention time of the drugs as the changes were within 2% RSD thus indicating the robustness of the method.

System suitability: System suitability measures the performance of the chromatography system in relation to the method. The parameters were calculated based on six injections of a fixed concentration of each drug and were found to be conforming to the reference values demonstrating that the method has been optimized for analysis in the present RP-HPLC system.

Table 3: System suitability parameters

Parameter (limits)	PCM	%RSD	TH	%RSD
Retention time	3.141	0.04	3.461	0.09
Tailing factor (<2)	1.097	1.28	1.856	1.7
Theoretical plates (>2000)	8745	3.69	6007	3.71
Resolution in relation to previous peak (>2)	-	-	2.136	1.56

Conclusion

The RP-HPLC method developed for the simultaneous quantitative estimation of PCM and TH has been found to be rapid, economical, linear, precise, and robust during validation. The ease of mobile phase preparation and the short run times ensure proper utilization of time and resources. The method can be used for the analysis of drug content both in pure standard and in pharmaceutical dosage forms.

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