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Bioanalytical Method Development and Validation for Simultaneous Determination of Paracetamol, Tramadol HCL, Domperidone Tablet Formulation by RP-HPLC: Its Pharmacokinetic Applications

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Research Article

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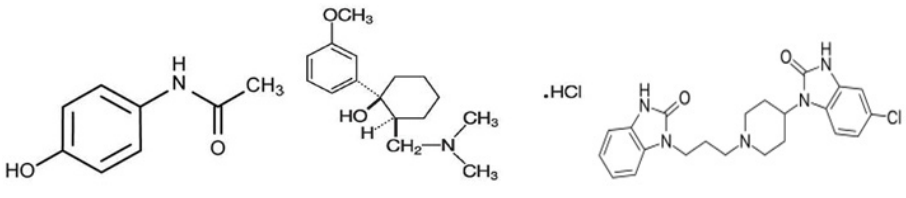
ABSTRACT

Paracetamol (PAR), Tramadol HCl (TRM) and Domperidone (DOM) used for acute musculoskeletal pain. Method of quantification of low level of these drugs in biological samples is needed to be optimized. Chromatographic separation was achieved on using C18 column by mobile phase composed of Methanol: Phosphate buffer (50:50 v/v) at a flow rate of 1 ml/min and UV detection at 272 nm. In-vivo pharmacokinetic study, blood samples were collected from rats after oral administration of Paracetamol, Tramadol HCl and Domperidone (1 mg/kg). The retention time for Paracetamol, Tramadol HCl and Domperidone was found to be 3.3, 5.3 and 8.9 min, respectively. The results of analysis were validated statically. The linearity range of PAR, TRM and DOM was 250-1500, 25-150 and 25-150 µg/mL, recovered amount of drugs by accuracy was 100.12, 99.87 and 100.4% and tablet analysis was 99.88, 100.28 and 99.6%, respectively. The method was successfully applied to the oral pharmacokinetic study for PAR, TRM, DOM. The maximal concentration (C_{max}) of Paracetamol, Tramadol HCl and Domperidone being 156.36, 335.15 and 55.34 (ng/mL) at (T_{max}) 3, 4 and 6 and half-life found at 3.75, 5.8 and 4.8 hours, respectively. The current method has been successfully applying to the pharmacokinetic study.

INTRODUCTION

Chemically, paracetamol **(Figure 1a)** is N-(4-hydroxyphenyl) acetamide. It is an analgesic antipyretic agent. It is effective in treating mild to moderate pain such as headache, neuralgia and pain of musculo- skeletal origin [1]. The half-life of Paracetamol 1-3 hours and bioavailability is 70-90%. Chemically, Tramadol HCl **(Figure 1b)** is 2-(dimethylaminomethyl)-1-(3- methoxyphenyl) cyclohexanol. Tramadol hydrochloride is a central analgesic property used for treating moderate to severe pain. Tramadol hydrochloride works by binding to the µ-opioid receptor and by acting as a serotonin-norepinephrine reuptake inhibitor. Tramadol HCL is agonist µ- opioid receptor [2]. The half-life of Tramadol HCl is 6.3-7.4 hours and bioavailability are 70-75%. Domperidone **(Figure 1c)** is chemically 5-chloro-1-[1-[3-(2-oxo-1, 3-dihydrobenzoimidazol-1-yl) propyl]-4-piperidyl] -1, 3- dihydrobenzoimidazol-2-one. DOM is a specific blocker of dopamine receptor. It speeds gastrointestinal peristalsis, causes prolactin release and is used as antiemetic and tool in the study of dopaminergic mechanism **(Figure 1)** [2,3].



(a) Paracetamol (b) Tramadol HCl (c) Domperidone

Figure 1. Chemical structure of a. Paracetamol b. Tramadol HCl and c. Domperidone.

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The PAR, TRM and DOM determination has been done by HPLC [4] and Stability indicating method has been reported [5,6]. The PAR was determined by Sub-Acute Toxicity Studies of Paracetamol Infusion in Albino Wistar Rats [7]. The bioanalytical method development and validation and its pharmacokinetic applications not available for these three drugs. The most widely accepted guidelines for method validation is the ICH and USFDA guidelines Q2 (R1), which is used both in pharmaceutical and medical science [8-11].

EXPERIMENTAL

Instrumentation

HPLC (Water 600 controller) instrument equipped with a model code 6CE In Line Degasser AF, Reciprocating pump, Rheodyne 7725i manual injector with a 20 µl fixed loop and HPLC syringe of 100UI and with UV-Vis detector. Separation and quantitation were made onRP-18, Inertsil ODS column (250 × 4.6 mm × 5 µ) (5 µm particle size). The detector was set at 254 nm. The pH of mobile phase was checked by HANNA pH meter.

Material and Method

PAR, TRM and DOM were received from Glenmark Pharmaceutical LTD, Sinner, India. Dipotassium hydrogen phosphate, Sodium dihydrogen phosphate and Sodium chloride used were of AR grade and HPLC grade. Methanol used was of HPLC grade. The plasma was taken from the laboratory animals.

Optimization of Method

The isocratic separation of compounds was carried out by using mobile phase composed of Methanol: Phosphate buffer (Dibasic) pH 4 (50:50 v/v), at a flow rate 1 ml min^{-1}. The injection volume was fixed at 20 µl. The mobile phase was filtered through membrane filter (0.45 µ). The reason for selection of this method is that, it shows good resolution than the other mobile phases. The method was accepted in this chromatographic condition **(Figure 2)**.

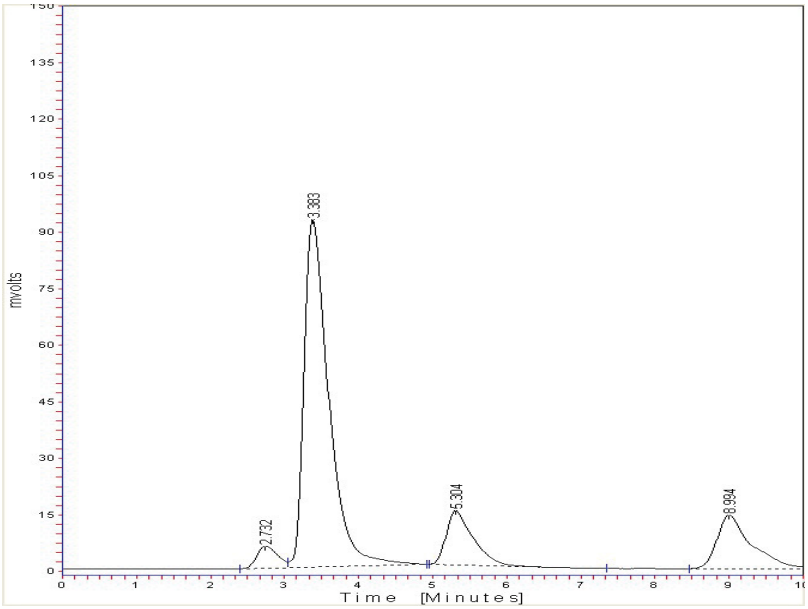

Figure 2. Representative Chromatograph of Paracetamol, Tramadol HCl and Domperidone in Spike Plasma. Preparation of Standard Solutions.

The stock solution for PAR, TRM and DOM were prepared by weighing each 10 mg in 100 ml of methanol. The further dilutions were prepared in mobile phase to obtain the working standard solution of PAR, TRM and DOM in the ranges of 2.5-12.5 µg/ml, 0.25-1.25 µg/ml and 0.25-1.25 µg/ml.

Preparation of Plasma

The stored rat plasma samples were allowed to thaw at room temperature before processing and centrifuged at 4000 rpm for 10 min, an aliquot (0.5 ml) was pipette into a 10 ml polypropylene tube and Acetonitrile (2.0 ml) was added. The mixture was vortex mixed briefly and after standing for 5 minutes at room temperature the mixture was centrifuged at 4000 rpm for 5 min. The supernatant was carefully transferred into tube and injected into HPLC system. Resultant samples were injected in developed chromatographic conditions.

Application of the Assay

The above-mentioned method was successfully used to analyze plasma samples of PAR, TRM and DOM for a pharmacoki-

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netic study. The PAR, TRM and DOM found in tablets by the application of the assay are shown below. The % RSD for precision is<2 which confirms that method is sufficiently precise **(Figure 3)**.

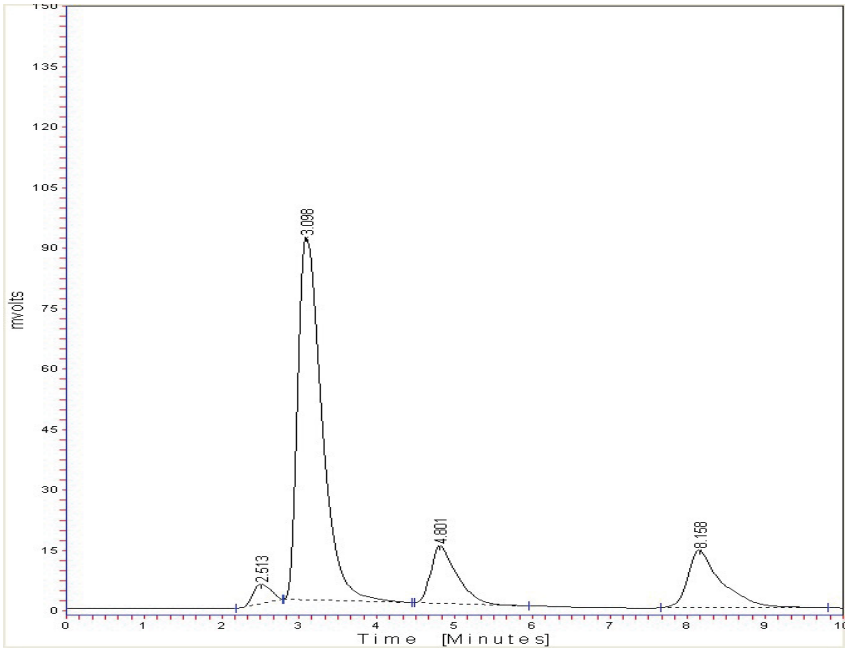

Figure 3. Representative Chromatograph of Tablet Analysis of Paracetamol, Tramadol HCl and Domperidone in Spike Plasma.

VALIDATION

Specificity

The ability of the bioanalytical method to measure and differentiate the analyte in the presence of components that may be expected to be presented. The PAR, TRM and DOM used for bioanalytical method development and validation and this method were accepted in chromatographic condition for the bioanalytical study **(Figure 4)**.

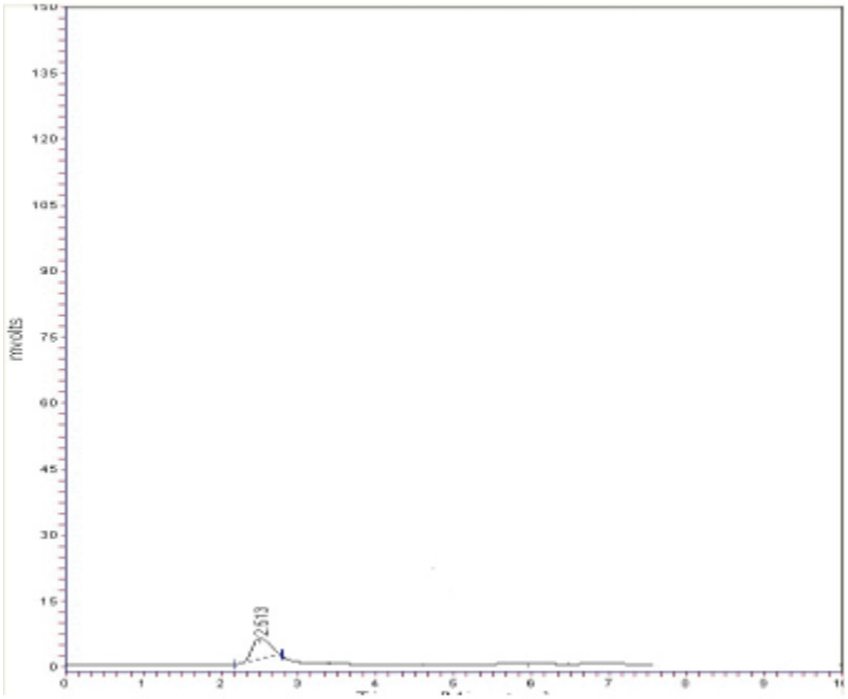

Figure 4. Representative Chromatogram of blank plasma Linearity.

The calibration curves over the concentration ranges of 2.5-12.5, 0.25-1.25 and 0.25-1.25 µg/mL for PAR, TRM and DOM showed a good linearity, where all correlation coefficients exceeded 0.999. The results had shown that within the concentration range, there was an excellent correlation between peak area ratio against corresponding concentration of PAR, TRM and DOM shown in **Table 1**.

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Table 1. Result of Linearity.

Parameter	PAR	TRM	DOM
Linearity range	2.5-12.5 µg/ml	0.25-1.25 µg/ml	0.25-1.25 µg/ml
Regression equation	y=51.96x – 25.4	y=4.88-3.33	y=13.6-523
Correlation coefficient (r2)	0.999	0.999	0.999
Slope	51.96	4.88	13.6
Y-Intercept	25.4	-3.33	-5.23

Accuracy

Percent recovery from plasma was used for determination of accuracy of the proposed method. Percentage recovery was determined at three concentration levels 400, 500, 600 µg/mL, 40, 50, 60 µg/ml and 10, 15, 20 µg/ml for PAR, TRM and DOM when drugs in plasma were injected. The % recovery and % RSD were calculated shown in **Table 2**.

Table 2. Result of Accuracy study.

Conc. (µg/mL)			Std. drug added (µg/mL)			Recovered amount (µg/mL)			% Recovery		
PAR	TRM	DOM	PAR	TRM	DOM	PAR	TRM	DOM	PAR	TRM	DOM
500	50	15	400	40	10	399.9	39.5	9.8	100.08	99.75	100.3
500	50	15	500	50	15	501.1	50.2	15.5	100.45	100.06	100.4
500	50	15	600	60	20	599.5	59.1	20.8	99.85	99.8	100.5

Precision

Repeatability and inter-day precision studies was performed by injecting three replicates in HPLC column. The relative standard deviation was below 2.0% in all tested concentrations are shown in **Table 3.**

Table 3. Result of Precession.

Validation Parameter	% Mean			S.D.			% R.S.D		
	PAR	TRM	DOM	PAR	TRM	DOM	PAR	TRM	DOM
Repeatability	100.2	100.73	100.18	0.1	0.945	0.256	0.099	0.93	0.257
Day to Day	99.83	100.1	100	0.251	0.3257	0.19	0.252	0.325	0.19
Analyst to Analyst	100.14	100.08	100.05	0.075	0.476	0.1761	0.074	0.475	0.1760

Robustness

Robustness was assessed by making deliberate changes in the chromatographic conditions as per ICH guidelines. The method was investigated under a condition of changes in pH of mobile phase (pH 4 ± 0.1). It shows in **Table 4.**

Table 4. Result of Robustness.

Variation In Mobile Phase pH	% Mean			S.D.			% R.S.D		
	PAR	TRM	DOM	PAR	TRM	DOM	PAR	TRM	DOM
(pH 3.9)	100.27	101.03	100.21	0.29	0.98	0.542	0.28	0.97	0.541
(pH 4.1)	100.15	100.65	100.41	0.1616	1.15	0.720	0.1613	1.14	0.717

Pharmacokinetic Studies

The animal study was approved by the Institutional Animal Ethical Committee of the Amrutvahini College of Pharmacy, Sangamner (Approval Number: CPCSEA/1153/PO/AC/08). The validated RP-HPLC method was successfully applied to the pharmacokinetic study of PAR, TRM and DOM in rats **(Table 5)**. Wistar rats (250-300) were housed with free access to food and water. A pharmacokinetic study on the drug was performed healthy female subjects (n=9). The blood samples (1 mL) were collected by retro orbital puncture after overnight fasting of animals the mixture following oral administration of 32.5, 37.5 and 10 mg tablet of Paracetamol, Tramadol HCl and Domperidone at pre-dose 0, 1, 2, 3, 4, 6, 8, 12, 24h, in EDTA collection tubes. The tubes were centrifuged at 3000 rpm for 10 min at 4°C and the plasma was collected. Immediately after collection, the plasma samples were subjected to flash-freezing and stored at -70°C till their use. Plasma samples with the drug and processed as per the liquid-liquid extraction procedure described earlier. Plasma concentration- time data of Paracetamol, Tramadol HCl and Domperidone were analyzed by non-compartmental method using Kinetica version 5.0(Thermo scientific). The described method was applied to a pharmacokinetic study in rats. After a single oral administration of PAR, TRM and DOM (1 mg/kg) to rats, plasma concentrations were determined over a period of 24 h after administration. The mean plasma concentration–time curves after an oral administration of PAR, TRM and DOM (1 ng/ml) is shown in **Figure 5** and the pharmacokinetic parameters were summarized in **Table 6**. The C_{max} of PAR, TRM and DOM were obtained from the curves, that was 156.36, 335.15 and 55.15 ng/ mL respectively, and the T_{max} was 3, 4 and 6 hrs. shown in **Figure 6**.

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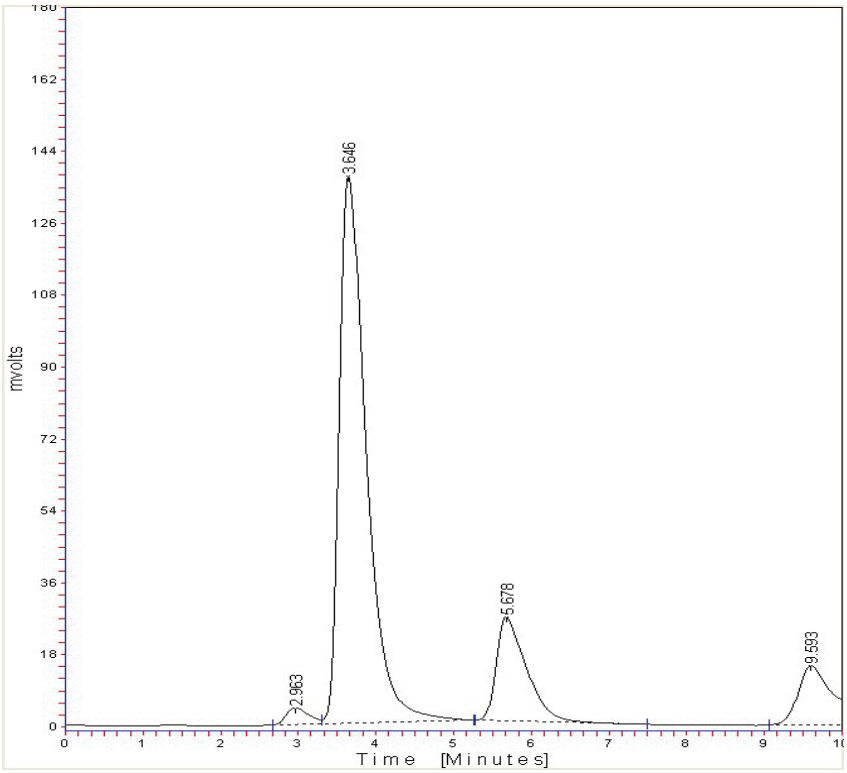

Figure 5. Representative Chromatograph of Paracetamol, Tramadol HCl and Domperidone in Spike Rat Plasma.

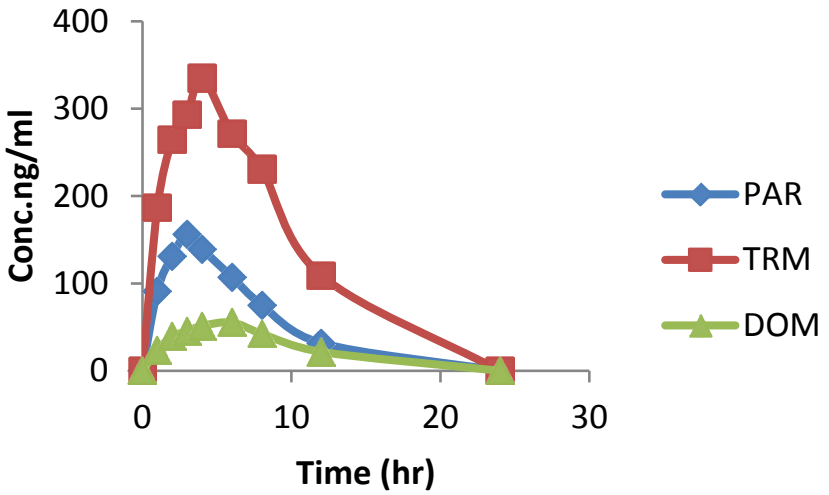

Figure 6. Mean Rat Plasma Concentration-Time Profiles of Paracetamol, Tramadol HCl and Domperidone.

Table 5. Assay of Tablet Analysis.

Parameters	TRADOL-PD		
	PAR	TRM	DOM
Mean (%)	99.88	100.28	99.6
S.D.	0.09165	0.4386	1.5307
R.S.D. (%)	0.09176	0.4373	1.5368

Table 6. Pharmacokinetic Studies.

Parameters	PAR	TRM	DOM
Cmax (ng/mL)	156.36	335.15	55.34
Tmax (hrs)	3	4	6
AUC0-last (ng/ml.h)	1072552500	2742600000	469687500
AUC0-tot (ng/ml.h)	1251699667.3	3821515685.2	640304279
AUCextra(ng/ml.h)	179147167.3	1078915685.2	179147167.3
t1/2 (hrs)	3.75	5.8	4.8

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CONCLUSION

In the present study, a simple, accurate, and precise method was developed for the simultaneous estimation of Paracetamol, Tramadol HCl and Domperidone by RP-HPLC. The developed method was composed of methanol and phosphate buffer (pH 4) as mobile phase. The developed method was specific, which shows no interference of blank matrix with the quantification and also shows short elution i.e.<10 min for Paracetamol, Tramadol HCl and Domperidone. Administration of Paracetamol, Tramadol HCl and Domperidone had no significantly effect on single oral dose. The developed method was applied successfully for pharmaco-kinetic studies of PAR, TRM and DOM in rats. The calculated Pharmacokinetic parameters include that the biological half-life ($t_{1/2}$) of drug is prolonged in rat by oral dose. The T_{max} value considerably high. Maximum plasma concentration (C_{max}) of the optimized oral dose found to be less. The method has several applications for bioequivalence, bioavailability and drug interaction studies.

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