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## Research Article

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### Analytical Method Development and Validation of Simultaneous Estimation of Tramadol, Paracetamol, Domperidone in Tablet Dosage Form by RP-HPLC

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#### ABSTRACT

A simple reverse phase high pressure liquid chromatography (RP-HPLC) method has been developed and validated for simultaneous estimation of tramadol paracetamol and domperidone in tablet dosage forms. The compounds were separated on inertsil C-18 ODS column with mixture of phosphate buffer (pH-6.0 adjusted with o-phosphoric acid): methanol in the ratio 80:20v/v at flow rate 0.8ml/min. UV detection was 278nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The developed and validated method was successfully used for analysis of TRAM-PD tablets. Total run time for analytes was nearly 10min with Tramadol, Paracetamol and Domperidone eluting with retention time 6.2, 3.7, 7.7 mins respectively. Validation studies showed the method is specific, reliable and reproducible. Calibration plots were linear over the concentration range 3.75-11.25µg/ml for tramadol, 32.5-97.5µg/ml for paracetamol and 1-3µg/ml for domperidone. The LOD and LOQ were 0.36, 5.44, 0.08 and 1.08, 16.49, 0.24 respectively.

**Keywords:** RP-HPLC, Octa Decyl Silane, Tramadol HCl, Paracetamol, Domperidone

#### ARTICLE INFO

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## 1. Introduction

Tramadol HCl is chemically (TMD: (+/-) cis-2-[(dimethyl amino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydro chloride). It is centrally acting analgesic, having agonist action at the  $\mu$ - opioid receptor and affects reuptake at the noradrenergic and serotonergic system. Paracetamol is chemically N- (4-Hydroxy phenyl) acetamide. It is a centrally and peripherally acting non- opioid analgesic and anti-pyretic. It is also used in the management of severe pain. Domperidone is chemically 5-Chloro-1-[1-[3-(2-oxo-2, 3-dihydro-1H-benzimidazole-1-yl) propyl]-4-piperidinyl]-1, 3-dihydro-2H-Benzimidazol-2-one. It has peripheral dopamine receptor ( $D_1$ & $D_2$ ) blocking properties which increase gastric motility and peristalsis; therefore, facilitating gastric emptying and decreasing small bowel transit time. It is prescribed for management of emesis. These three drugs are widely prescribed either in single or in combination for various disease conditions. Tramadol and paracetamol combination is widely prescribed by the physicians to avoid pains and domperidone is prescribed

## 2. Materials and Methods

### Instrumentation

Analysis was carried out using Waters HPLC coupled with PDA detector on Inertsil ODS C-18 column (250\*4.6mm). Packed with 5microns size as stationary phase.

### Drugs and Chemicals

Tramadol HCl, Paracetamol and Domperidone were procured from Active Pharma Labs as gift samples. HPLC grade methanol was procured from Standard chemicals and Di-potassium hydrogen phosphate and HPLC grade water were procured from Merck Ltd., India.

### Preparation of diluents

HPLC grade methanol was prepared and degassed in ultrasonic water bath for 5 min.

### Preparation Of mobile phase

The mobile phase 0.02M Di-potassium hydrogen phosphate (PH-6.0 with O-phosphoric acid): methanol in the ratio of 80:20 was delivered in isocratic mode.

### Preparation of standard stock solution

Accurately weighed and transferred 18.75 mg of tramadol and 162.5 mg of Paracetamol and 5 mg of Domperidone

with paracetamol to control nausea, gastritis and reflux esophagitis. So it is highly needed to develop a simple method for simultaneous determination of these drugs in combination dosage forms. Paracetamol is official in Indian pharmacopoeia. This suggests titrimetric and UV spectroscopic method for paracetamol bulk and tablets. Domperidone is official in Indian pharmacopoeia where assay is described by titrimetric method. Tramadol is official in Indian pharmacopoeia. This suggests titrimetric method in bulk. Literature survey showed that various analytical methods like spectrometric, HPLC, GC have been reported for determination of tramadol, paracetamol<sup>1-3</sup> and either individually or combination with some other drugs. Many methods have been described in literature for the determination of domperidone<sup>4-7</sup>, paracetamol individually. The RP-HPLC method has been reported for estimation of Tramadol HCl, Paracetamol and Domperidone<sup>8,9</sup> in tablet dosage forms.

into 100ml clean dry volumetric flask and added about 70ml of diluents. It was sonicated to dissolve completely and made volume up to the mark with the same diluents stock solution (187.5, 1625, 50  $\mu$ g/ml). From this, 0.4ml of the solution was pipette into other 10ml volumetric flask and diluted with upto the mark with diluents (7.5, 65 & 2  $\mu$ g/ml).

### Preparation of Sample solution

Accurately weighed (weight equivalent to one tablet) and transferred 18.75 mg of tramadol and 162.5 mg of Paracetamol and 5 mg of Domperidone into 100ml clean dry volumetric flask and added about 70ml of diluents. It was sonicated to dissolve completely and made volume up to the mark with the same diluents stock solution (187.5, 1625, 50  $\mu$ g/ml). From this, 0.4ml of the solution was pipette into other 10ml volumetric flask and diluted with upto the mark with diluents (7.5, 65 & 2  $\mu$ g/ml).

## 3. Results and Discussion

### Chromatography

The chromatogram of best resolution and minimum tailing was obtained by RP-HPLC method using 0.02M buffer (Ph 6.0 adjusted with O-Phosphoric acid): methanol (80:20) as mobile phase. The Retention Time for Tramadol HCl, Paracetamol and Domperidone in optimized condition was observed to be 3.7, 6.2, 7.7 min.

The chromatogram shown in fig. 3

### Method Validation [10]

#### Accuracy & Precision

The accuracy & precision of method were determined and calculated as %RSD. The value of %recovery and %RSD showed in table 1 & 2. The value of %RSD in table shows that the method is accurate within acceptable limit of 2%.

#### Linearity

A least square regression analysis was performed on peak area of each drug vs its concentration in the range of 3.75-11.25  $\mu$ g/ml for tramadol, 32.5-97.5  $\mu$ g/ml for paracetamol and 1.0-3.0  $\mu$ g/ml for domperidone and correlation coefficient was found to be 0.999. A linear relationship was observed for all drugs shown in table 3.

#### Specificity

The specificity studies revealed the absence of any other excipient interference. The interaction study between the three drugs in the standard solution was carried out by comparing peaks of each drug individually with peaks obtained in drug mixture indicating that the analytes did not interact with each other.

#### LOD and LOQ

For determining the LOD and LOQ, the method based on Standard deviation and slope was adopted. Results were shown in table 4.

#### System Suitability Parameters

System suitability parameters as R.T, Peak asymmetry, Resolution, Theoretical Plates were calculated from data obtained from 3 replicates injections of mixed standard

solutions to check the system parameters. The results were shown in table 5.

#### Assay of Tablets

The value of analysis of tablets obtained by the method was between 99.0-99.8 which showed that the estimation of dosage forms were within acceptance level. Results was showed in table 6.

**Table 1:** Accuracy Results of Paracetamol, Tramadol, Domperidone

S.No	Conc µg/ml	Paracetamol			Tramadol HCl			Domperidone		
		Amount Added	A.Found	%Rec overy	Amount Added	A.Found	%Rec overy	Amount Added	A.Found	%Rec overy
01	50%	32.5	32.05	98.62	3.75	3.72	99.20	1	0.98	98.0
02	100%	65	66.10	101.69	7.5	7.60	101.33	2	1.99	99.5
03	150%	97.5	96.96	99.45	11.25	11.29	100.36	3	3.03	101.00

**Table 2:** precision data of Paracetamol, Tramadol, Domperidone

No.of injections	Paracetamol Peak Area	Tramadol Peak Area	Domperidone Peak Area
Injection:01	234876	249897	98597
Injection:02	238743	242376	99480
Injection:03	241321	239876	99453
Injection:04	232453	239913	99546
Injection:05	231389	246754	100567
Average	235756.40	243763.20	99528.60
Standard Deviation	4202.57	4427.95	699.02
%RSD	1.78	1.82	0.70

**Table 3:** Regression equation and correlation coefficient ( $r^2$ )

Drug Name	Regression Equation	$r^2$
Tramadol	Y=33189X	0.999
Paracetamol	Y=3563X	0.999
Domperidone	Y=49426X	0.999

**Table 4:** Results of LOD and LOQ results

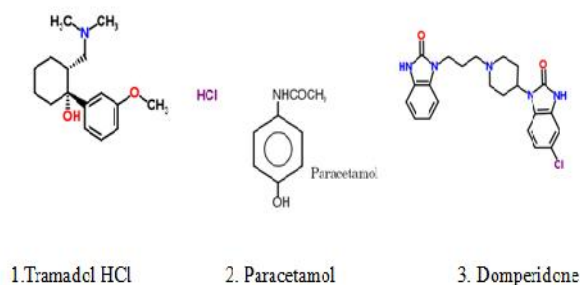
Drug Name	LOD	LOQ
Tramadol	0.36	1.08
Paracetamol	5.44	16.49
Domperidone	0.08	0.24

**Table 5:** Observation of system suitability parameters

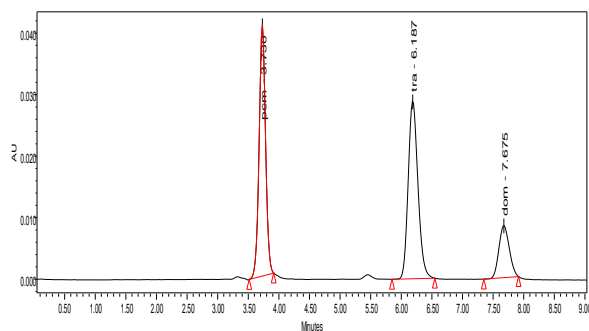
S.No	Parameter	Paracetamol	Tramadol	Domperidone
1	Retention Time	3.7	6.2	7.7
2	Resolution		9.8	4.75
3	Tailing Factor	0.98	1.15	1.16
4	Plate count	5733.4	7259.8	8833.3

**Table 6:** Assay Results

Formulation	Component	L.C	% Assay
Brand-A	Paracetamol	325mg	99.8
	Tramadol	37.5mg	99.0
	Domperidone	10mg	99.5



**Figure 1:** Structures of Tramadol, Paracetamol, Domperidone



**Figure 3:** Standard chromatogram of Paracetamol (R.T 3.7 min), Tramadol (R.T 6.2 min) Domperidone (R.T 7.7 min)

## 5. Abbreviations

RP-HPLC - Reverse Phase High Pressure liquid chromatography

ODS - Octa Decyl Silane

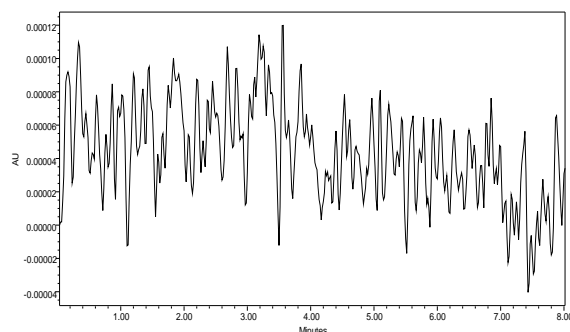
UV - Ultra violet

LOD - Limit Of detection

LOQ - Limit of Quantification

TMD - Tramadol HCl

GC - Gas Chromatography



**Figure 2:** Chromatogram of blank

## 4. Conclusion

The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation. So the proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Tramadol HCl and Paracetamol & Domperidone in tablet dosage form.

PDA Detector - Photodiode Array Detector

Min - Minutes

% RSD - Relative Standard Deviation

Con. - Concentration

µg/ml - microgram per milli liters

$r^2$  - Coefficient of variation

R.T - Retention Time

L.C - Lable Claim

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