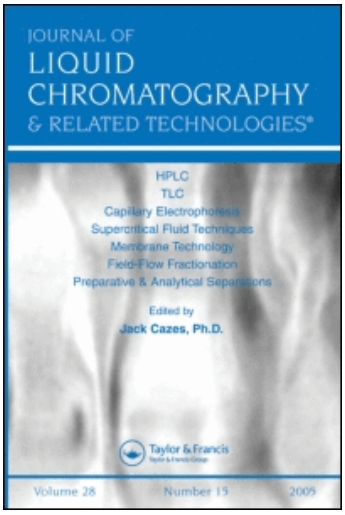

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SIMULTANEOUS ESTIMATION OF TRAMADOL HYDROCHLORIDE, PARACETAMOL AND DOMPERIDONE BY RP-HPLC IN TABLET FORMULATION

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□ *A new high performance liquid chromatography method was developed and validated for the quantitation of tramadol, paracetamol, and domperidone in pharmaceutical formulations. Determination was performed using a Inertsil-ODS-3 (C_{18}) column (5 μm, 250 mm × 4.60 mm), a mobile phase containing methanol–phosphate buffer (pH 4.0; 40+ 60, v/v), in gradient flow rate 1.2 mL. The method was validated with respect to linearity, precision, robustness, and accuracy. The calibration graphs ranged from 250 to 1500 mg/mL for paracetamol, 25 to 150 mg/mL for tramadol, and 5 to 30 mg/mL for domperidone. Intra- and interday relative standard deviation values for the standard solutions were 0.077%, 0.98%, and 1.04%, respectively. Repeatability of relative standard deviation values was 0.115%, 0.494%, and 1.97% respectively. Total recoveries of paracetamol, tramadol, and domperidone from the laboratory prepared mixtures were 100.4, 100.06, and 100.2%, respectively.*

Keywords domperidone, HPLC, paracetamol, RP-HPLC, simultaneous estimation, tarmadol

INTRODUCTION

Paracetamol (*N*-(4-hydroxyphenyl) ethanamide) is commonly prescribed for the relief of mild to moderate pain and as an antipyretic. It is rapidly absorbed from the gastrointestinal tract and is primarily metabolized by conjugation with glucuronic and sulphuric acid. Paracetamol is rapidly and uniformly distributed into most body tissues. About 25% of acetaminophen in blood is bound to plasma proteins and plasma half-life of 1.25–3 hours.[2–5] Tramadol hydrochloride (±)-cis-2[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclo-hexanol hydrochloride is a centrally acting

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analgesic with efficacy and potency ranging between weak opioids and morphine. The drug acts as an opiate agonist by selective activity at the μ-opioid receptors. In addition to opiate agonist activity, tramadol inhibits reuptake of norepinephrine and serotonin, which appears to contribute to the drug's analgesic effect. Tramadol is rapidly and almost completely absorbed after oral administration, but its absolute bioavailability is only 65–70% due to first-pass metabolism.[1] Domperidone 1,3-dihydro-5-chloro-1-(1-(3-(2,3-dihydro-2-oxo1H-benzimidazol-1-yl)propyl)-4-piperidinyl)-2H-benzimidazol-2-one is a dopamine antagonist that produces extrapyramidal reactions. It stimulates gastrointestinal motility and is used as an antiemitic for the short term treatment of nausea and vomiting in various aetiologies, including that associated with cancer therapy including nausea and vomiting associated with levodopa or bromocriptine therapy for Parkinsonism.[1,2,4]

Tablet dosage forms containing PAR, TRM, and DOM in ratio of 500 mg:50 mg:10 mg of various brands are available in the market. PAR has been reported to be determined by HPLC[6,7] from formulations and in biological fluids. Ratio spectra derivative spectrophotometry,[8] TRM determination has been done by HPLC.[9,10] DOM determinations have been reported by HPLC[11] and the stability indicating assay method.[12] However, there is no method available for the simultaneous determination of these three drugs. Therefore, an attempt was made to develop a new, rapid, and sensitive method for the simultaneous determination of PAR, TRM, and DOM. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH norm, which is mandatory also.[13,14]

EXPERIMENTAL

Materials

Paracetamol was received from Lupin Lab. Ltd. (Bhopal, India). Tramadol Hydrochloride was received from Alembic Ltd. (Vadodra, India) and Domperidone was received from Cadila Health Care (Ahemdabad, India). Sodium hydroxide and hydrogen peroxide were purchased from S.D. Fine-Chem Ltd. Methanol was procured from Merck India Ltd. (Mumbai). All other chemicals were of analytical grade.

Instrumentation

The pH of the mobile phase was checked on a microprocessor waterproof pH tester (pH tester 20, eutech instruments, oakton, USA). The overall illumination at the point of placement of samples was 6000 lux, which was tested using a calibrated lux meter (Lutron LX-102 digital

light meter, Marcucci S.P.A, vignate, Milan). The HPLC system, equipped with a LC-10ATVP pump, and SPD-10ATvp data were acquired and processed using CLASS-VP software (all from Shimadzu, Kyoto, Japan).

Chromatographic Conditions

Initially, a reverse phase LC separation was tried using methanol and water (50:50) as mobile phase, in which domperidone retained at 25 min, although other two drugs responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of TRM and PAR. To improve the tailing factor, the pH of mobile phase becomes an important factor. Thereafter, methanol–phosphate buffer of pH 4.0 in the ratio of 60:40 v/v was selected to improve resolution and the tailing for the three peaks were reduced considerably and brought close to 1. At 60% phosphate buffer all three drugs get properly resolved but domperidone eluted at 12.5 min and at 40% phosphate buffer retention time for domperidone to 6.19 min, but paracetamol and tramadol gets merged. Therefore a gradient was planed and concentration of buffer was maintained to 60% till the elution of paracetamol and tramadol and then reduced to 40% for early elution of domperidone. The problem of the merging of paracetamol with the buffer peak is solved by reducing the flow rate at 0.8 mL/min.

Considering the overlay spectra of these three drugs, 273 nm seems to be the most suitable detection wavelength. As at 245 nm (λ_{max} of PAR) the absorbance of both the drugs domperidone and tramadol is negligible

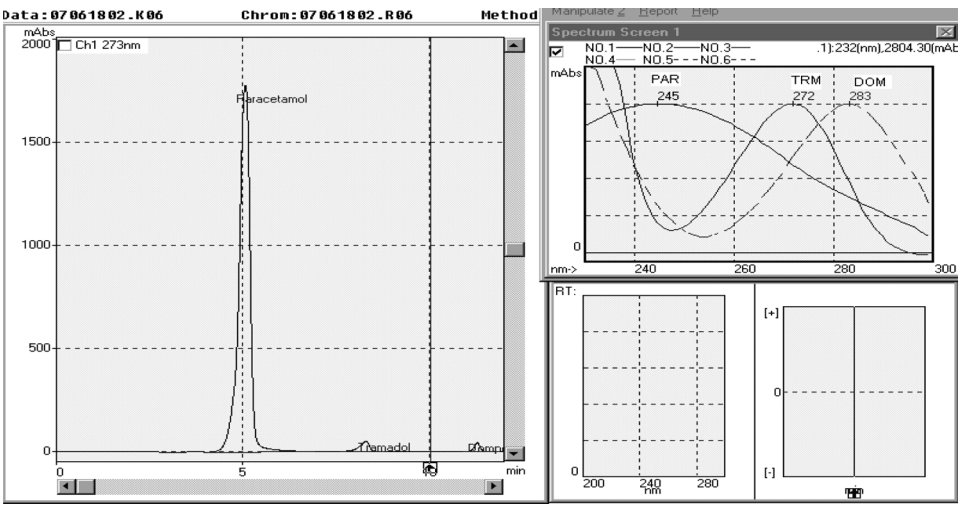

FIGURE 1 Representative chromatogram of paracetamol, tramadol and domperidone.

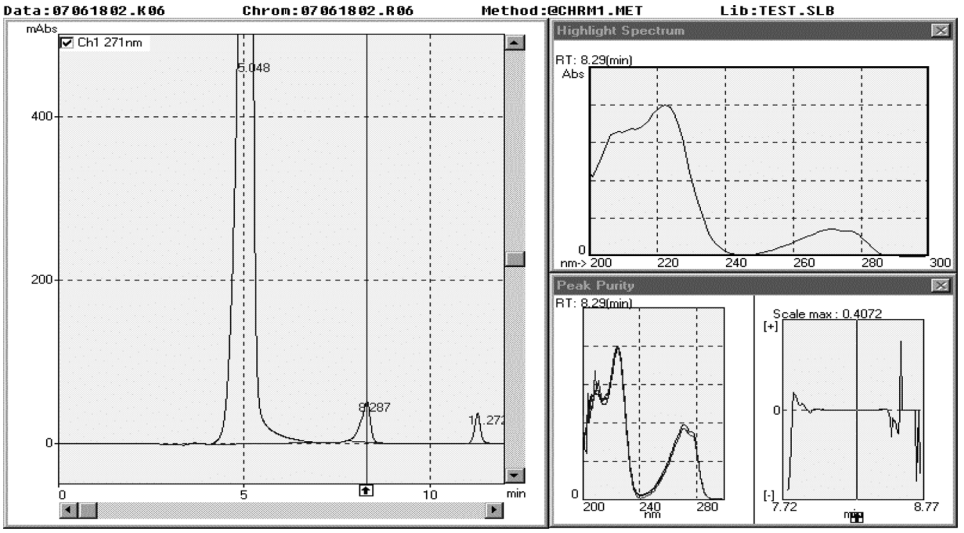

FIGURE 2 Representative chromatogram of paracetamol, tramadol and domperidone (by minimizing the scale).

(valley) and at 283 nm, the λ_{max} of domperidone, response of tramadol is fairly good but that of paracetamol is less. Therefore, at 273 nm tramadol shows maximum absorbance. Also, absorbance of the remaining two drugs are satisfactory.

The concentration of TRM and DOM is low, hence the AUC is not noticeable in comparison to PAR, and therefore its peak is not clearly visible on the same scale in chromatogram (Fig. 1). By minimizing the scale, the peak corresponding to TRM and DOM is clearly visible (Fig. 2).

RESULTS AND DISCUSSION

System Suitability

System suitability parameters such as the number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for PAR, TRM, and DOM were 1989, 6254, and 22125, respectively.

TABLE 1 Results of System Suitability

Sr. no.	Parameters	PAR	TRM	DOM
1	No. of theoretical plates	1989	6254	22125
2	HETP	0125	.0399	0.0112
3	Tailing factor	0.89	0.95	1.02

TABLE 2 Results of Recovery Study

	Conc. of drug in preanalyzed samples (μg/mL)			Std. drug sol. added (μg/mL)			Recovered amount* (μg/mL)			Recovered (%)		
Sr. no.	PRA	TRM	DOM	PRA	TRM	DOM	PRA	TRM	DOM	PRA	TRM	DOM
1	1500	150	30	500	50	5	196.27	19.96	3.96	101.1	100.7	101.2
2	1500	150	30	750	75	10	220.46	22.74	4.50	99.07	102.6	102.2
3	1500	150	30	1000	100	15	245.05	24.84	5.02	100.3	99.59	101.6
Mean										100.2	100.8	101.69
S.D										1.06	1.31	0.520
RSD (%)										1.06	1.29	0.512

*Mean of three readings.

Linearity

PAR, TRM, and DOM showed a linearity of response between 250–1500, 25–150, and 5–30 $\mu g\,mL^{-1}$, respectively. The linearity was represented by a linear regression equation as follows.

$$Y\ (PAR)\ = 24804\,conc. - 23957\ (r^2 = 0.9999)$$
$$Y\ (TRM) = 5856.2\,conc. - 30990\ (r^2 = 0.9994)$$
$$Y\ (DOM) = 17355\,conc. - 649.13\ (r^2 = 0.9992)$$

Accuracy

Recovery studies were performed to validate the accuracy of the developed method. To the preanalyzed sample solution, a definite concentration of the standard drug was added and recovery was studied. These results are summarized in Table 2.

TABLE 3 Results of Precision

		Mean* (%)			S.D.			R.S.D. (%)		
Sr. no.	Validation parameter	PRA	TRM	DOM	PRA	TRM	DOM	PRA	TRM	DOM
1	Repeatability	100.09	100.4	100.5	0.11	0.49	1.2	0.11	0.49	1.9
2	Intermediate precision day to day	100.1	100.3	99.14	0.077	0.98	1.03	0.077	0.98	1.04
3	Intermediate precision analyst to analyst	100.1	100.1	100.5	0.18	0.21	1.2	0.17	0.20	1.28

*Mean of fifteen determinations (3 replicates at 5 concentration level).

TABLE 4 Results of Robustness

Sr. no.	Validation parameter	Mean* (%)			S.D.			R.S.D. (%)		
		PRA	TRM	DOM	PRA	TRM	DOM	PRA	TRM	DOM
1	Robustness (pH-3.5)	100.2	100.3	100.1	0.070	0.41	0.51	0.070	0.41	0.51
2	Robustness (pH-4.5)	100.02	101.01	100.6	0.072	0.21	1.94	0.072	0.20	1.92

*Mean of six determinations.

Precision

Five dilutions in three replicates were analyzed in the same day for repeatability, and results were found within acceptable limits (RSD < 2), as shown in Table 3. Five dilutions in three replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation. Although the RSD value for DOM is higher than that of PAR and TRM because of its low concentration, but than all results also fell within acceptable limits (RSD < 2), as shown in Table 3.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase is methanol:phosphate buffer (pH-4.0) in 40:60 V/V to 40:60 V/V to methanol:phosphate buffer (3.5) in 45:55 V/V to 55:45 V/V and methanol:phosphate buffer (pH-4.5) in 40:60 V/V to 40:60 V/V in gradient flow. Results of the analysis are summarized in Table 4.

Tablet Analysis

Contents of PAR, TRM, and DOM found in the tablets by the proposed method are shown in Table 5. The low values of R.S.D. indicate that the method is precise and accurate.

TABLE 5 Results of Tablets

Sr. no.	Parameters	TRAM-PD		
		PRA	TRM	DOM
1	Mean* (%)	99.99	100.39	100.33
2	S.D.	0.171	0.452	2.30
3	R.S.D. (%)	0.170	0.451	2.22

CONCLUSION

An RP-HPLC method was developed and validated for simultaneous estimation of PAR, TRM, and DOM in tablet dosage form. The proposed method is fast, accurate, and precise; hence, it can be employed for routine quality control of tablets containing these three drugs in industry.

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