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Simultaneous RP-high performance liquid chromatographic determination of paracetamol, tramadol hydrochloride and domperidone in pharmaceutical formulations

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ABSTRACT

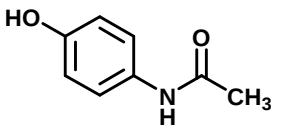
A simple, sensitive and accurate reversed phase high performance liquid chromatographic method has been developed for detection and quantitative determination of Paracetamol, Tramadol hydrochloride and Domperidone. Methyl paraben was used as internal standard. Efficient chromatographic separation was achieved on a Betasil C8 column (125mm x 4.6 mm, 5 µm) using with a 85:15 *(v/v)* mixture of 0.02% Trifluroacetic acid in water, 0.02% Trifluroacetic acid in acetonitrile. The chromatographic conditions were optimized to avoid interference from excipients as well as to achieve acceptable resolution between three drug components with internal standard Methyl paraben. The developed method was validated for linearity, accuracy, precision, limit of quantitation and robustness.

KEYWORDS

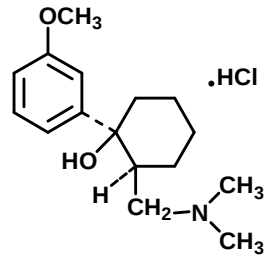
Column liquid chromatography;
Paracetamol;
Tramadol hydrochloride;
Domperidone.

INTRODUCTION

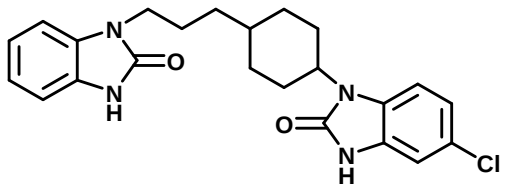
Paracetamol *para*-acetylaminophenol,is analgesic drug. Tramadol (±)cis-2-[(dimethylamino)methyl]-1-(3-methoxy phenyl)cyclohexanol hydrochloride, is anti-inflammatory drug. and Domperidone 6-Chloro-3-[3-(2-oxo-3H-benzimidazole-1-yl]piperidin-4-yl]1H-benzimidazole-2-one is a dopamine antagonist and is used as antiemetic drug. The structures of these three drugs are shown in Figure 1[1,2]. This combination is used as anti-emetic and pain associated with gastrointestinal disorders. One combination of these drugs, which contains 325mg Paracetamol, 37.5mg Tramadol hydrochloride and 10mg Domperidone. The literature survey revealed that simultaneous determination of Paracetamol, Tramadol hydrochloride and Domperidone with inter-



1) Paracetamol



2) Tramadol hydrochloride



3) Domperidone

Figure 1 : The structure of paracetamol tramadol hydrochloride and domperidone.

nal standard by HPLC is not reported. However, references are available for their individual quantitation[3-5] The objective of the present work is to develop a simple, fast and accurate HPLC method for simultaneous determination of Paracetamol, Tramadol hydrochloride and Domperidone from pharmaceutical formulation.

EXPERIMENTAL

Chemicals and reagents

Standards were obtained from reputed research centres. Tramazac PD tablets were procured from the market. Acetonitrile, Methanol used were HPLC grade and Trifluroacetic acid was AR grade. Double distilled water was used throughout the work. All dilutions were performed in standard volumetric flasks.

Preparation of stock and working standard solutions

A stock solution of Paracetamol (1630µg mL^{-1}) was prepared by dissolving about 81.5mg Paracetamol (99.44%) in methanol in standard 50ml volumetric flask (solution A). A stock solution of Tramadol hydrochloride (375µg mL^{-1}) was prepared by dissolving about 37.5mg Tramadol hydrochloride (99.80%) in methanol in standard 100ml volumetric flask (solution B). A stock solution of Domperidone (100µg mL^{-1}) was prepared by dissolving about 20mg Domperidone (99.80%) in methanol in standard 200ml volumetric flask (solution C). Internal standard (IS-Methyl paraben) stock solution (200 µg mL^{-1}) was prepared by 20 mg Methyl paraben in methanol in a 100ml standard volumetric flask(solution D). Solutions containing mixture of Paracetamol, Tramadol hydrochloride and Domperidone at five different concentrations were prepared in the mobile phase and Methyl paraben was added to each as internal standard (10 µg mL^{-1}).The concentration ranges for each of three drugs in the working standard solutions were 97.5 µg mL^{-1} – 227.5µg mL^{-1} for Paracetamol, 11.25µg mL^{-1} – 26.25 µg mL^{-1} for Tramadol hydrochloride and 3µg mL^{-1} - 7 µg mL^{-1} for Domperidone.

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed in to a homogeneous powder and a quantity equivalent to one tablet (750 mg) was weighed in a 100mL volumetric flask, dissolved in methanol, and filtered through Whatman no. 41 filter paper. The filtrate (5ml) was quantitatively transferred to a 100mL volumetric flask, 10mL of internal standard solution was added to it, and diluted up to the mark with mobile phase.

Instrumentation and chromatographic conditions

Chromatography was performed with a Merck Hitachi pumpL-7100, a Merck autosampler L-7250,and a Merck Diode array detector L-7455. Chromatograms and data were recorded by means of HSM software. Compounds were separated on a 125mm x 4.6mm, i.d.,5 µm particle, Betasil C_8 column. The mobile phase was 0.02% Trifluroacetic acid in water: 0.02% Trifluroacetic acid in acetonitrile in the ratio (85:15)v/v. The flow rate was 1.0mL min^{-1}. Twenty microlitres of sample was injected and detection wavelength was 210nm for determination of three drugs. A typical HPLC chromatograms obtained from simultaneous determination of Paracetamol, Tramadol hydrochloride and Domperidone from pharmaceutical formulation are shown in Figure 2.

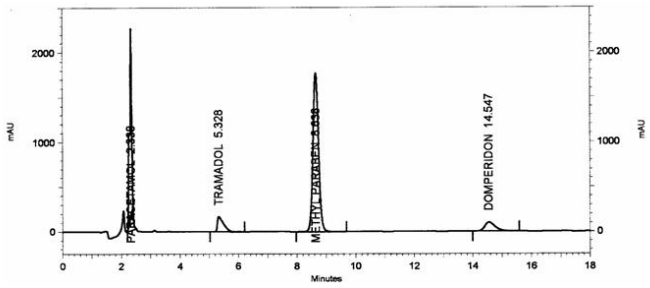


Figure 2 : HPLC chromatogram obtained during simultaneous determination of paracetamol[1], tramadol[2], and domperidone[4] with methyl paraben[3] as internal standard.

RESULT AND DISCUSSION

Method validation

The chromatographic conditions were optimized to obtain good baseline separation and peak shape.

Linearity

Linearity was evaluated by analysis of working standard solutions of Paracetamol, Tramadol hydrochloride and Domperidone of five different concentrations[3].

The linear ranges from 97.5 μg mL^{-1} – 227.5μg mL^{-1} for Paracetamol, 11.25μg mL^{-1} – 26.25 μg mL^{-1} for Tramadol hydrochloride and 3μg mL^{-1} - 7 μg mL^{-1} for Domperidone. The drug to internal standard peak area ratio and concentration of each drug were subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the three pharmaceuticals are listed in TABLE 1. The results shows that within these concentration ranges there was excellent correlation between peak area ratio and concentration of each drug.

TABLE 1 : Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r) n = 5
Paracetamol	0.157	-0-391	0.9997
Tramadol hydrochloride	0.011	-0.002	0.9999
Domperidone	0.047	-0.002	0-9997

Limit of detection and quantification

The limit of detection (LOD) and quantification (LOQ) were established at signal to noise ratios of 3:1 and 10:1, respectively. The LOD and LOQ of Paracetamol, Tramadol hydrochloride and Domperidone were determined experimentally by injecting each drug six times. The LOD for Paracetamol, Tramadol hydrochloride and Domperidone were 0.04 μg mL^{-1}, 0.5 μg $mL^{-1}$and 0.08 μg mL^{-1} respectively. The LOQ for Paracetamol, Tramadol hydrochloride and Domperidone were 0.12 μg mL^{-1}, 1.5 μg $mL^{-1}$and 0.25 μg mL^{-1} respectively.

Precision

System precision was verified using mixed standard solutions, which was analysed six times and RSD of Paracetamol, Tramadol hydrochloride and Domperidone peak were evaluated and found to be within 2%. Precision of the method was studied for repeatability. Repeatability was demonstrated by analyzing six separate Paracetamol, Tramadol hydrochloride and Domperidone tablets sample solution at 100% of the test concentrations. The RSD were found to be within 2%.The results are given in TABLE 2.

TABLE 2 : Results of precision

	Paracetamol	Tramadol HCl	Domperidone
Mean amount of drug found (mg per tablet)	326.78	37.69	9.99
% Assay	100.55	100.52	99.88
%RSD	0.56	0.33	0.54

System suitability

System suitability tests were performed in accordance with USP 24/NF 19 to confirm the reproducibility of the equipment was adequate for the analysis to be performed. The test was performed by injecting 20μLof mixed solution of drug and internal standard. The RSD values were found to be satisfactory and meeting the requirements of USP24 NF19 (RSD less than 2 %). Theoretical plates, resolution, tailing factor were determined and presented in TABLE 3.

TABLE 3 : Results for system suitability

Parameters	Paracetamol	Tramadol hydrochloride	Methyl paraben	Domperidone
Theoretical plates	2158	2543	2847	3354
Tailing factor	1.15	1.55	1.08	1.04
Resolution	-	3.45	3.87	7.51

Accuracy

The accuracy of the method was determined by measuring the recovery of the drugs by the method of standard additions. Known amounts of each drug corresponding to 100,110,120 and 130% of the label claim for each drug were added to preanalysed sample, to

TABLE 4 : Result for recovery

	Level	Initial Conc In mg	Amount added in mg	Total Conc mg	Amount of drug found in (mg)	Recovery (%)
Paracetamol	100%	325	0	325	326.32	100.41
	110%	325	32.5	357.5	357.12	99.89
	120%	325	65.0	390	389.53	99.88
	130%	325	97.5	422.5	419.40	99.27
Tramadol hydrochloride	100%	37.5	0	37.5	37.42	99.78
	110%	37.5	3.75	41.25	41.17	99.92
	120%	37.5	7.5	45	45.55	100.21
	130%	37.5	11.25	48.75	48.56	99.63
Domperidone	100%	10	0	10	10.05	100.54
	110%	10	1	11	10.98	99.85
	120%	10	2	12	12.02	100.18
	130%	10	3	13	13.05	100.38

determine whether the excipients present in the formulation led to positive or negative interferences[3]. Each set of additions was repeated three times. The accuracy was expressed as the percentage of the analytes recovered by the assay; the results obtained are listed in TABLE 4. The results indicate the method enables highly accurate for simultaneous determination of Paracetamol, Tramadol hydrochloride and Domperidone

Robustness

The robustness of a method is a measure of its capacity to remain unaffected by small variations in method conditions; it provides an indication of the reliability of the method during normal application. The robustness of the proposed method was evaluated by altering composition of the mobile phase and different columns from different manufacturers- the experimental conditions were changed and the chromatographic characteristics were evaluated.

Mobile phase composition was changed by ±10% of organic solvent. Both the ends of the mobile phase composition variations in the resolution of Paracetamol and Tramadol, Tramadol and Methyl paraben and Methyl paraben and domperidone and the retention time of the three drugs with internal standard were within 2%. Column to column variation was also determined using same make but different lot no. C_8 column the results indicated there were no significant differences between the two columns, and that separation of the three active substances is achievable under the given conditions using the method developed which is satisfactory for the simultaneous determination of Paracetamol, Tramadol hydrochloride and Domperidone in the formulation.

CONCLUSION

The proposed isocratic HPLC method for simultaneous detection and quantitation of Paracetamol, Tramadol hydrochloride and Domperidone in tablets is highly sensitive, accurate and precise. This procedure can be easily adopted for the routine quality control analysis of tablet dosage form. The method was validated for its performance parameters such as linearity, precision, accuracy, LOD, LOQ.

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