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SIMULTANEOUS ESTIMATION AND VALIDATION OF IBUPROFEN AND TRAMADOL IN TABLET DOSAGE FORMS USING RP HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of Ibuprofen and Tramadol in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 30 volumes of ammonium acetate buffer, 40 volumes of acetonitrile and 30 volumes of Methanol with detection of 238 nm. Linearity was observed in the range 60-140 µg/ml for Ibuprofen (r^2 =0.999) and 6-14 µg /ml for Tramadol (r^2 =0.996) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Liquid chromatography (LC), RSD Relative standard deviation, r^2 correlation coefficient

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions. By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product

meet two critical requirements. Established identity and purity and Established bio availability/dissolution. Analytical chemistry- A branch of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis).The techniques commonly used are titration, precipitation, spectroscopy, chromatography, etc. Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time." A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it. A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species.

Ibuprofen, a propionic acid derivative, is a prototypical nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties. The exact mechanism of action of ibuprofen is unknown. Ibuprofen is a non-selective inhibitor of cyclooxygenase, an enzyme invovled in prostaglandin synthesis via the arachidonic acid pathway. Its pharmacological effects are believed to be due to inhibition cylooxygenase-2 (COX-2) which decreases the synthesis of prostaglandins involved in mediating inflammation, pain, fever and swelling. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation. Inhibition of COX-1 is thought to cause some of the side effects of ibuprofen including GI ulceration. Ibuprofen is administered as a racemic mixture. The R-enantiomer undergoes extensive interconversion to the S-enantiomer *in vivo*. The S-enantiomer is believed to be the more pharmacologically active enantiomer. Ibuprofen is a nonsteroidal anti-inflammatory agent (NSAIA) or nonsteroidal anti-inflammatory drug (NSAID), with analgesic and antipyretic properties. Ibuprofen has pharmacologic actions similar to those of other prototypical NSAIAs, which are thought to act through inhibition of prostaglandin synthesis.

A narcotic analgesic proposed for moderate to severe pain. It may be habituating. Tramadol is also prepared as a variable release capsules, marketed under the brand name ConZip. For example, a 150 mg capsule will contain 37.5 mg of the immediate release form and 112.5 mg of the extended release form. Tramadol and its O-desmethyl metabolite (M1) are selective, weak OP3-receptor agonists. Opiate receptors are coupled with G-protein receptors and function as both positive and negative regulators of synaptic transmission via G-proteins that activate effector proteins. As the effector system is adenylate cyclase and cAMP located at the inner surface of the plasma membrane, opioids decrease intracellular cAMP by inhibiting adenylate cyclase. Subsequently, the release of nociceptive neurotransmitters such as substance P, GABA, dopamine, acetylcholine and noradrenaline is inhibited. The analgesic properties of Tramadol can be attributed to norepinephrine and serotonin reuptake blockade in the CNS, which inhibits pain transmission in the spinal cord. The (+) enantiomer has higher affinity for the OP3 receptor and preferentially inhibits serotonin uptake and enhances serotonin release. The (-) enantiomer preferentially inhibits norepinephrine reuptake by stimulating alpha(2)-adrenergic receptors (1, 2).

Aim is to develop new RP HPLC method for the simultaneous estimation of ibuprofen and tramadol pharmaceutical dosage form

MATERIALS AND METHODS

Determination of Working Wavelength (λmax) (3-7)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Ibuprofen

10 mg of IBUPROFENwas weighed and transferred in to 100ml volumetric flask and dissolved in water and then make up to the mark with water and prepare 40 µg /ml of solution by diluting 4ml to 10ml with water.

Preparation of standard stock solution of Tramadol

10 mg of TRAMADOL was weighed in to 100ml volumetric flask and dissolved in water and then dilute up to the mark with water and prepare 30 µg /ml of solution by diluting 3ml to 10ml with water.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of ibuprofen and 10 mg of tramadol in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of ibuprofen and tramadol is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains tramadol-05 mg ibuprofen-50 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of tramadol and ibuprofen (µg/ml) were prepared by dissolving weight equivalent to 10 mg of tramadol and ibuprofen and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of tramadol and ibuprofen was made by adding 1 ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The isobestic point was found to be 285 nm for the combination. The amount of ibuprofen and tramadol present in the taken dosage form was found to be 100.25 % and 101.34 % respectively (Table-1).

Table -1 Assay Results

	IBUPROFEN		TRAMADOL	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	506899	515556.000	931844	947876.000
Injection-2	514450.000	508489	950651.000	962290
Injection-3	512714	513449.000	958312	960335
Injection-4	513898	513770	958137	972115
Injection-5	513154.000	516509	948997.000	969359.000
Average Area	512223.000	513554.600	949588.2	962395
Assay(%purity)	100.259965		101.348669	

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The % RSD for the retention times and peak area of ibuprofen and tramadol were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-2 and 3).

Table-2 Results for system suitability of ibuprofen

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.004	516853	11284.978	1.748
2	2.004	518006	11236.126	1.754
3	2.003	515590.000	11389.440	1.746
4	2.006	524462.000	11400.617	1.775
5	2.006	520400	11480.903	1.765
Mean	2.005	519486.000	-	-
SD	0.001	519132.833	-	-
%RSD	**0.06**	3135.268	-	-

Table -3 Results for system suitability of tramadol

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	6.187	339373	31694.107	1.780
2	6.166	338536	31994.193	1.770
3	6.194	340815	31465.607	1.766
4	6.182	341269	31677.251	1.749
5	6.182	342995	31620.098	1.765
Mean	6.1822	340597.6	-	-
SD	0.010305	1731.719	-	-
%RSD	0.166694	0.508436	-	-

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150% (Fig-1-3).

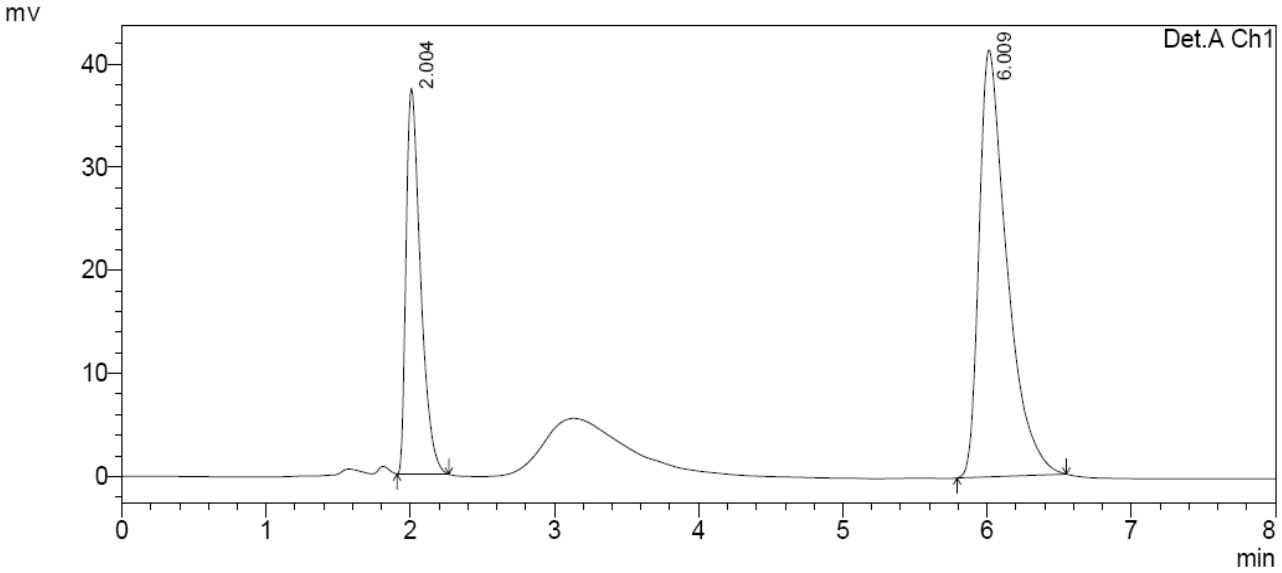

Fig-1 Chromatogram of 50% recovery

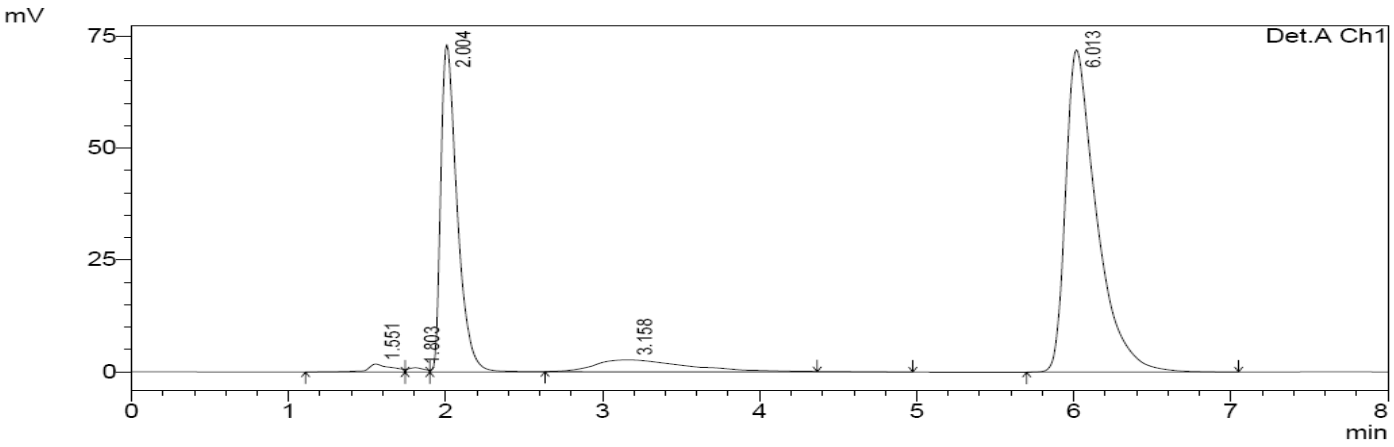

Fig-2 Chromatogram of 100% recovery

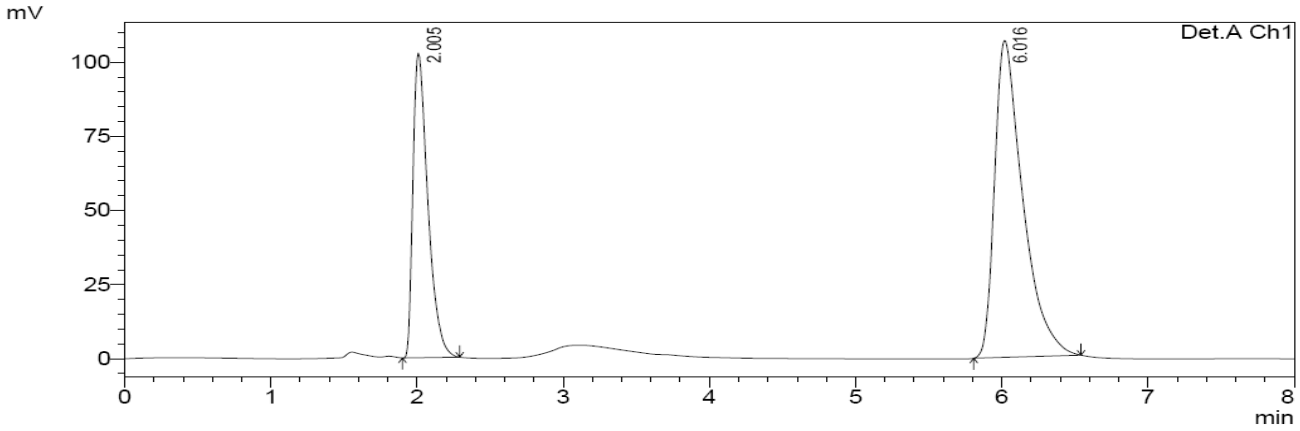

Fig-3 Chromatogram of 150% recovery

The percentage mean recovery of ibuprofen and tramadol is 99.19 % and 99.89 % respectively. Test results for tramadol and ibuprofen are showing that the %RSD of Assay results are within limits. The results were shown in table Table-4.

Table-4 Results for method precision of ibuprofen and tramadol

IBUPROFEN		
S.No.	Rt	Area
1	2.005	517461.000
2	2.004	517192.000
3	2.005	518753.000
4	2.007	521539.000
5	2.006	521945.000
6	2.006	521320.000
avg	2.0055	519701.667
stdev	0.0010	2156.215
%RSD	**0.05**	**0.41**

TRAMADOL		
S.No.	Rt	Area
1	6.015	991225
2	6.013	992250
3	6.013	994414.000
4	6.014	1016768.000
5	6.013	1012833
6	6.013	1019377.000
avg	6.014	1004477.833
stdev	0.001	13185.450
%RSD	**0.01**	**1.31**

From the observation it was found that the system suitability parameters were within limit at all variable conditions. The observation between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

Newly developed method for the simultaneous estimation Ibuprofen and Tramadol was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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