pKa and Partition coefficient Determination of Montelukast sodium by Spectrophotometric and HPLC Technique

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Abstract

The acid dissociation constant (pKa) of a pharmaceutical drug is a significant physicochemical parameter that impacts biopharmaceutical properties of drugs and other chemical characteristics. Determination of pKa and Partition coefficient of Montelukast Sodium by spectrophotometric technique and HPLC technique individually. From the measurement structure plan of dosage form and pharmaceutical examination point of view, the pKa and Partition coefficient are the most significant physicochemical properties of pharmaceutical drug which should be assessed. The pKa estimation of Montelukast Sodium was done by utilizing UV-Visible spectrophotometric strategy and Partition coefficient was evaluated utilizing HPLC technique. Experimental estimation value of pKa1 of Montelukast Sodium was found to be 3.3 and pKa2 of Montelukast Sodium was found to be 4.4. Two graphical techniques were utilized to estimate the acid dissociation constant (pKa) for Montelukast Sodium and the Partition coefficient of Montelukast Sodium by HPLC method was experimentally estimated to be 2.42.

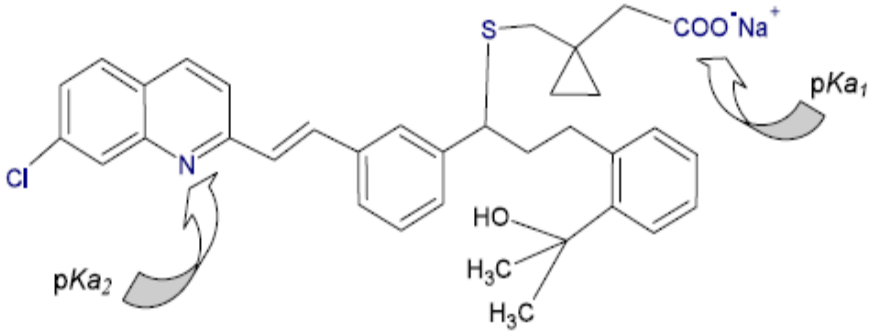
Keywords: Montelukast sodium, Active pharmaceutical ingredient(API),Design of Buffers, Spectrophotometric Titration, High Performance liquid Chromatography (HPLC), dissociation constant(pKa), Partition coefficient (Log P).

INTRODUCTION

Montelukast sodium is a selective and orally active leukotriene receptor antagonist which is being utilized in the treatment of asthma. It has a place with astyrylquinolines series that repress the cysteinyl leukotriene CysLT1receptor[1].

Montelukast sodium is portrayed synthetically as 2-[1-[[(1R)-1-[3-[2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl) acetic] cyclopropyl] sulfanylmethyl] propyl]phenyl acid} monosodium salt[2]. (Fig.1)

Fig.1. Chemical structure of Montelukast Sodium[3].



Montelukast attach with high fondness and selectivity to the CysLT1 receptor Montelukast hinders physiologic actions of LTD4 at the CysLT1 receptor with no agonist activity. Literature survey study reveals that Montelukast sodium is evaluated exclusively by U.V spectrometry, RP-UPLC, estimationof related substance by RP-HPLC, LC/MS/MS, UPLC-MS/MS, UV-HPLC, and Assay by HPLC[5].

Ionization state of a pharmaceutical drug substance with respect to the pH of a solution is determined by its acid dissociation constant, pKa and Partition coefficient. From the pharmacokinetic perspective the pKa and log P Partition coefficient turns out to be much progressively huge if there should be an occurrence of poorly water soluble drugs. Log P are the cardinal factors which must be considered in the pharmaceuticals investigational analysis and evaluation of dosage form design of the drug .While anticipated pKa and Log P values of Montelukast sodium arereported, exhaustivepursuit of literature survey did not reveal strong evidence regarding experimental estimation of pKa and Log P (partion coefficient) values of Montelukast Sodium.

We carried out two experiments , one for pKa determination and other for log P (partition coefficient) estimation by spectrophotometric technique and HPLC procedure individually.

Literature survey data uncovers that there are no spectrophotometry methods for the estimation of pKa montelukast sodium.For this reason, we have planned a down to earth approach to decide the pKa, which requires just the utilization of a spectrophotometer

The principle points of interest of this technique are that it is sensitive touchy, takes a brief time, and the equipments are both inexpensive and easy tooperate i.e. user-friendly.An alternative option to potentiometric titration is UV–VIS spectrophotometry because of the fact that it can deal with compounds with lower solubility and lower sample concentrations. The fundamental benefit of this technology that it is higher sensitivity ($>10^6$ M) to compounds with favourable molar absorption coefficients. The largest change in absorbance occurs at the pH corresponding to a pKa value. These progression changes are normallyrecognized from the first derivative of the absorbance against time plot or from overlay plots of the different spectra. The estimation of pKa values by UV–VIS assumes that the solute of interest is pure. Customarily, spectral data at a single analytical wavelength are acquired from a sample in a series of buffer solutions with known pH values, after which the pKa is determined. To utilize this strategy, the absorption spectra of individual species must be characterized previously and the molar absorptivities of protonated and deprotonated species are subsequently required. These estimations are non-trifling if acid-base equilibria comprise more than two ionization steps or if reacting components are not stable within two pH units of the pKa value, so a multi-wavelength spectrophotometric approach has been developed to determine acid dissociation. Target-factor examination has been applied to deduce pKa values from the multi-wavelength UV absorption data recorded at different pH esteems values.

pKa values determination(Acid Dissociation Constant)

The estimation of value of the acid dissociation constant (pKa) is an important parameter that indicates the degree of ionizationof molecules in solution at different pH values[6]. Many chemical, physical and biological properties of naturaland synthetic compounds are governed by the interactions of acidic and basic groups.[7]

AcidDissociation constant, pKa, of a molecule decides it ionization state with respect to pH. pKa of drug straightforwardly influences pharmacokinetic properties like ADME.,its absorption, distribution, metabolism and excretion (ADME) profile. Ionized form of a drug molecule is more aqueous soluble but less membrane layer permeable.The acid–base dissociation constant of substances (pKa value) is a very important parameter in drug design and optimization. The degree of ionization strongly affects solubility and permeability[8].

EXPERIMENTAL

Materials and Methods:

Materials:

Montelukast Sodium was obtained as gift sample. Sodium hydroxide was obtained from SD.Fine Chemicals limited(Mumbai, India).Isopropyl alcohol, Disodium hydrogen phosphate and ortho-phosphoric acid (85% pure),Acetonitrile, Trifluroacetic acid,

Potassium dihydrogen phosphate, Methanol and Octanol was received from Merck (Mumbai, India). Milli-pore water was used throughout the study.

U.V and HPLC Instruementation and analytical conditions:

The pH of the buffer solutions were determined using digital pH meter (Metrohm, Model No 780). pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, 10.0 at room temperature. The spectra and absorbance readings (Spectrophotometric study) were conducted on UV Spectrophotometer (UV-24500 Shimadzu) operated at a wavelength range of 200–400 nm,centrifuge apparatus(Thermo Fisher, Model No 20059684).

A waters HPLC system containing e2695 seperation module.Using water 2996 photodiode array with a wavelength range of 190-800 nm sensitivity with operating software Empower-2 were used during the study.The detectorA zorbex Eclipse plus Phenyl-Hexyl, 4.6 mmx5 cm; 1.8 um particle size column was used for chromatographic analysis.Eluent-A Buffer(1.5 mL of Trifluroacetic acid added into 1 Litre water and Eluent-B Buffer(1.5 mL of Trifluroacetic acid added into 1 Litre Acetonitrile were used as mobile phase.238 nm was used as a detection wavelength.Flow maintained at 1.2mL/min.The column oven temperature was maintained at 30°C and autosampler temperaturewas maintained at 25°C ,at injection volume 10 ul.The diluents used is a mixture of Methanol and water in the ratio 90:10 v/v.The HPLC gradient program was as follows: Time (min)/A(v/v):B(v/v);T 0.01/60/40,T3/60/40,T16/49/51 with equilibrium time 7 min.

DETECTION METHOD

Method for pKa determination by UV-VIS Spectrophotometry:

The determination of pKa by spectrophotometric method is based on the principle that ionisation of the acidic or basic compound is pH dependent. Hence with change in pH of the solution the ratio of ionised form to the unionised form changes. At definite wavelength the ionised and unionised forms have different absorptions.

Preparation of Buffer Solutions at different pH:

0.02M Disodium hydrogen phosphate buffer was prepared in water and series of buffers covering the pH range between 1.6 and 7.5 were set up and prepared by adjusting pH with diluted ortho phosphoric acid. The pH values of all solutions were measured on a Metrohm, Model No 780 pH meter equipped with combined glass electrode which was calibrated by using standard buffers at pH 4.0 and 7.0.

Following series of buffers Solutions at different pH were prepared listed in Table 1.

Table 1: Series of buffers Solutions at different pH i.e from 1.60 to 7.50 pH.

Sr.No.	pH of BufferSolution	Sr.No.	pH of BufferSolution
1	1.60	11	4.11
2	1.80	12	4.30
3	2.00	13	4.58
4	2.20	14	4.88
5	2.40	15	5.20

6	2.80	16	5.40
7	3.00	17	5.99
8	3.20	18	6.50
9	3.40	19	7.01
10	3.90	20	7.50

Sample stock solution preparation (5mg/mL):

Weigh accurately about 100 mg of Montelukast Sodium sample into a 20ml volumetric flask added about 3ml of water and sonicated to dissolved and then make up the volume up to the mark with Isopropyl alcohol and mix well. From this stock solution, samples were prepared in different series of buffers by adding 50µL of stock sample solution, 5mL of Isopropyl alcohol and 15mL of respective buffer and scanned at 200nm to 400nm by utilizing every one of the twenty buffer solutions. These working solutions were then analysed under UV Spectrophotometer to measure their respective particular absorbance at 336nm and 373nm and absorbance at 284nm and 351nm as depicted by (Table-1) and (Table-2) respectively.

NOTE: All the solutions were kept in glass containers at 25 °C,shielded and protected from direct exposure of light.

Table -2 Absorbance estimation value of Montelukast Sodium at 336 and 373 nm in individual buffer solutions of pH 1.6–7.5.

pH	**Absorbance Value at wavelength(λ) 336nm**	**Absorbance Value at wavelength (λ) 373nm**
1.60	0.206	0.683
1.80	0.282	0.865
2.00	0.260	0.727
2.20	0.275	0.644
2.40	0.316	0.611
2.80	0.373	0.473
3.00	0.391	0.437
3.20	0.436	0.496
3.40	0.435	0.39
3.90	0.443	0.337
4.11	0.462	0.346
4.30	0.456	0.372
4.58	0.437	0.233

4.88	0.432	0.216
5.20	0.438	0.173
5.40	0.45	0.173
5.99	0.457	0.145
6.50	0.437	0.134
7.01	0.454	0.138
7.50	0.443	0.135

Table -3 Absorbance estimated value of Montelukast Sodium at 284 and 351 nm in individual cradle arrangementsof buffer solutions of pH 1.6–7.5.

pH	**Absorbance at wavelength (λ) 284nm**	**Absorbance at wavelength (λ) 351nm**
1.592	0.288	0.333
1.809	0.297	0.340
2.000	0.307	0.343
2.199	0.321	0.355
2.402	0.344	0.367
2.804	0.398	0.400
3.004	0.420	0.414
3.200	0.43	0.432
3.409	0.464	0.472
3.899	0.492	0.481
4.110	0.472	0.476
4.290	0.479	0.479
4.608	0.482	0.479
4.870	0.496	0.468

5.202	0.495	0.466
5.388	0.489	0.457
5.949	0.507	0.481
6.509	0.491	0.467
7.000	0.505	0.479
7.502	0.481	0.457

HPLC METHOD FOR LOG P (PARTITION COEFFICIENT) DETERMINATION:

For the estimation of Log P of Montelukast Sodium the test framework was changed modified shake flask method to meet the prerequisite of OECD rule for testing of chemicals. For this reason we have utilized 1-octanol as organic phase and phosphate buffer (pH 6.8 at 20 to 25°C) as aqueous phase.900 ml of n-Octanol was saved for immersion for 24 hours with 100 ml of phosphate support pH 6.8 at 20 to 25°C.900 ml of phosphate cushion pH 6.8 was kept for saturation for 24 hours with n-Octanol at 20 to 25°C.

In the wake of permitting an adequate time the two phases were separated and each was collected in a bottle of the saturated phosphate buffer pH 6.8 / n-Octanol. After saturation known amount of drug was stacked into aqueous phase with the end goal that the convergence of our last concentration of our final dilution falls in the range of developed HPLC method. The Test was performed by taking 90 mL of saturated phosphate buffer pH 6.8 and 10 mL of Octanol added about 50mg of the montelukast sodium sample was dissolved. The two layers are separated by centrifugation at 4000RPM for 10minutes than allowed the vessel to equilibrate at the required temperature after centrifugation. Taken 5mL of aliquots of each phase in each of the duplicate vessel and transferred into 50mL and made up the volume up to mark and determine the concentration of the montelukast sodium sample in both layers. After the extraction at ambient temperature, the amount of montelukast sodium remaining in buffer was determined by HPLC.

Chromatographic condition for Partition coefficient determination by HPLC

Column: - Zorbex Eclipse plus Phenyl-Hexyl, 4.6 mmx5 cm; 1.8 µm P.N959941-912

Eluent A: - Add 1.5 mL of trifluoroacetic acid to 1 L of water.

Eluent B: - Add 1.5 mL of trifluoroacetic acid to 1 L of acetonitrile.

Gradient Time	Eluent A	Eluent B
0.0	60	40
3.0	60	40
16.0	49	51

Equilibrium time: - 7 min

Sample volume: - 10 µL

Flow Rate: - 1.2 mL/min

Detector: - UV 238 nm

Column temperature: - 30°C

Auto sampler temperature: - 25°C

Diluent: - Methanol and water (9:1)

Standard solution preparation (0.13mg/mL Montelukast Sodiumin diluent)

System Suitability requirements:Relative standard deviation(RSD) of 6 injections of standard solution should not more than 0.80%.

Sample solution preparation (0.13mg/mLin diluent)**:**

0.1mg/mL Montelukast sodium sample in Buffer pH-6.8 and n-octanol.

Test conditions for Partition coefficient determination:

The following procedure should be followed:

Before Pow is determined, the two solvents used need to be mutually saturated. The procedure to the saturation is as following:

The test must be done in a constant temperature of 20 to 25°C.

900 ml of n-Octanol was kept for saturation for 24 hours with 100 ml of phosphate buffer pH 6.8. 900 ml of phosphate buffer pH 6.8 was kept for saturation for 24 hours with n-Octanol.

After allowing a sufficient time for the two phases are separated and each is collected in a bottle of the saturated phosphate buffer pH 6.8 / n-Octanol.

Test was performed by taking 90 mL of saturated phosphate buffer pH 6.8 and 10 mL of Octanol added about 50mg of the montelukast sodium sample was dissolved.

The two layers are separated by centrifugation at 4000RPM for 10minutes than allowed the vessel to equilibrate at the required temperature after centrifugation.

Taken 5mL of aliquots of each phase in each of the duplicate vessel and transferred into 50mL and made up the volume up to mark and determine the concentration of the montelukast sodium sample in both layers.

Calculation for partition coefficient

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water.

$$\text{Pow} = \frac{\text{Concentration (n - octanol)}}{\text{Concentration (water)}}$$

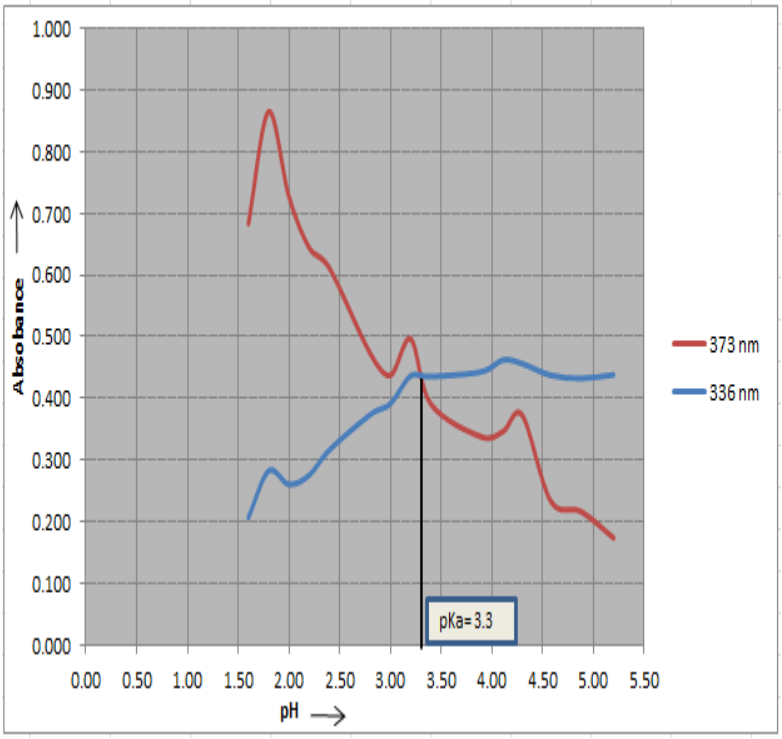
RESULTS AND DISCUSSION:

Experimental estimation of pka by pH metry is tedious and time consuming procedure, but by means of Spectrophotometry is easy & reproducible method.

The spectrophotometric measurements and absorbance measurements were performed by using a UV-Vis spectrophotometer in the wavelength range between 200 and 400 nm method for the pKa determination was opted by acknowledging the established fact that spectrophotometric determination of pKa produces most precise values. The pKa-1 of Montelukast Sodium was found to be 3.3(Fig-2) and pKa-2 of Montelukast Sodium was found to be 4.4.(Fig-3) Two graphical methods were used to estimate the acid dissociation constant (pKa) using absorbance measurements. The equation for the dependence of the absorbance on pH at λ = 336 nm and 373nm was obtained by using calibration curves.

The plot of the absorbance versus pH at these wavelength frequencies (λ = 336 nm and 373nm) is shown in Figure 2. The inflection point in the plot of the absorbance versus pH was observed at pH value 3.3 which was reflected as pKa-1value of Montelukast Sodium which is reflected in crossing point of the two direct linear bend curves as appeared in Figure 2.

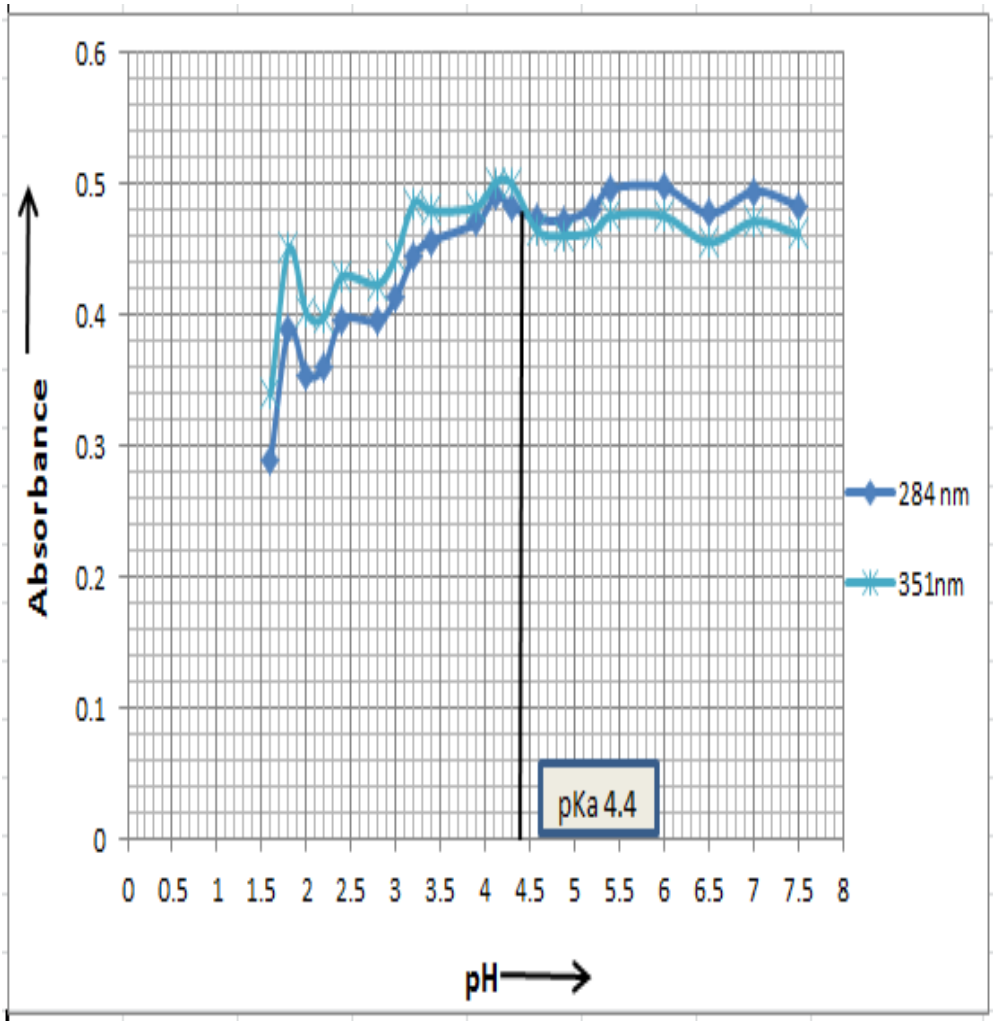
Figure -2. Absorbance bend curve graph among pH 1.60 to 7.50 and absorbance 373 nm and 336 nm



Montelukast Sodium in buffer solutions of pH 1.60 to 7.50 at wavelength frequency 373 nm and 336 nm. The point of intersection of graph depicted the pKa =3.3.

The plot of the absorbance versus pH at these wavelength frequencies (λ = 284 nm and 351nm) is shown in Figure 3. The inflection point in the plot of the absorbance versus pH was observed at pH value 4.4 which was reflected as pKa-2 value of Montelukast Sodium which is reflected in crossing point of the two direct linear bend curves as appeared in Figure 3.

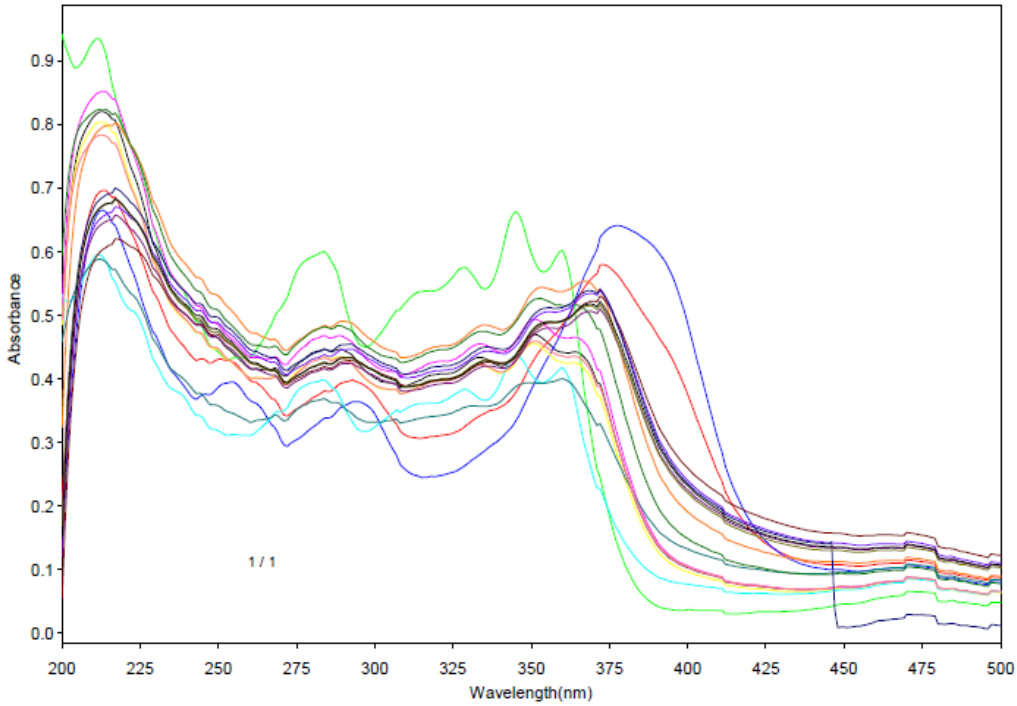
Figure -3. Absorbance bend curve graph among pH 1.60 to 7.50 and absorbance 284 nm and 351 nm.



Montelukast Sodium in buffer solutions of pH 1.60 to 7.50 at wavelength 284 nm and 351 nm. The point of intersection of graph depicted the pKa = 4.4.

As illustrated in Figure 4, Overlaid spectra shows the variation of absorbance peaks of Montelukast Sodium in various buffer solutions at different pHor increasing/decreasing of absorbance at particular wavelength at acidic and basic pH and on this basis four wavelengths were selected which were 284nm, 351nm, 336nm and 373nm.It is crystal clear reflected from the figure that thedrug exhibits pH dependent UV-absorption.

Figure -4. Overlaid Absorbance bend curves for the acidic, basic, and intermediate pH solutions.



Partition coefficient of montelukast sodium sample observed in both layers as follows:

Partition coefficient of Montelukast sodium sample	
Montelukast sodium sample	Partition Coefficient
Preparation –I	2.42
Preparation –II	2.42

CONCLUSION:

The information of a drug's physicochemical properties, to be specific the ionization constants, are vital in the advancement of new drug delivery approaches. Just because, it was tentatively decided the pKa estimations of the orally active leukotriene receptor antagonist which is being utilized in the treatment of asthma. The methodology explore UV–Visible spectrophotometry, a technique perceived to outfit pKa values with precision and reproducibility. In the pharmaceutical research zone, the ionization steady (pKa) is a physicochemical parameter significant since it impacts the dissolution capacity of active pharmaceutical ingredients and the route for pharmaceutical administration.

There was no proof accessible for affirming the experimental test pKa of Montelukast Sodium, we have utilized the inflection method as a fundamental report for determination of pKa of Montelukast Sodium .In inflection method strategy, we have plotted the absorbance of Montelukast Sodium drug solution against their corresponding individual pH and noticed the point of inflection of pKa value of Montelukast Sodium. A plot among Absorbance and pH for various cradles of buffers was drawn and it was obvious from the graph that deflection was found at pH 3.3 and 4.4 respectively.

For the first time the two unique strategies for pKa estimation of Montelukast Sodium dependent on the UV–Visible spectra were applied and established in the development of research. This research work revealed two pKa values for Montelukast Sodium, approximately, 3.3 and 4.4.

We recommend that partition coefficients, given the straightforwardness and recurrence with which they are estimated

experimentally, give another approach to benchmark in the advancement of new drug delivery approaches.

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