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MULTIVARIATE UV SPECTROPHOTOMETRIC QUANTIFICATION OF KETOROLAC TROMETHAMINE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The aim of this research work was to develop a simple, accurate, sensitive and validated Ultra Violet (UV) spectrophotometric assay using multivariate regression method for the analysis of Ketorolac. This multivariate calibration technique was based on equations constructed using linear regression analysis using the correlation between absorbance and concentration at five selected equidistant wavelengths. Ketorolac had a maximum absorbance of 322 nm. The findings were statistically analysed for significance. A linear plot in the concentration range of 7-13µg/mL, with a regression co-efficient of 0.999 was obtained. The % RSD for intra-day and Inter-day precision were 0.308 and 0.287, respectively. The assay was determined and found to be 99.10% - 99.70 % w/w.

Keywords: Ketorolac, antidiabetic agent, UV spectrophotometry, Multivariate calibration, Assay, ICH guidelines

INTRODUCTION

Ketorolac (KETO) **(Figure 1)** is a drug is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits analgesic activity in animal models. KETO is the tromethamine salt of ketorolac, a synthetic pyrrolizine carboxylic acid derivative with anti-inflammatory, analgesic and antipyretic properties. The primary mechanism of action responsible for ketorolac's anti-inflammatory, antipyretic, and analgesic

effects is the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX). Ketorolac is a non-selective COX inhibitor. It is considered a first-generation NSAID It is used by mouth, by nose, by injection into a vein or muscle, and as eye drops **[1]**. KETO chemically 2-amino-2-(hydroxymethyl) propane-1,3-diol;5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid. The chemical formula for KETO is C19H24N2O6, and molecular weight was found to be 376.4 g/mL **[2]**. KETO highly potent member of a new class of compounds having analgesic and anti-inflammatory activity **[3]**. Literature surveys reveal various methods as UV-Vis Spectrophotometry (UV) **[4-10]**, high performance liquid chromatography (HPLC) **[11-14]**, liquid chromatography and mass spectroscopy (LC-MS) **[15-16]**, high performance thin layer paper chromatography (HPTLC) **[17-18]**.

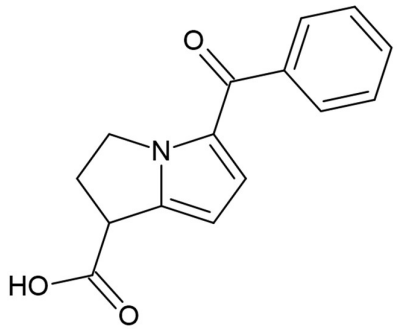

Figure 1: Chemical strucutre of Ketorolac

The suggested technique provides higher confidence in results as it directly evaluates KETO and has been attested with greater accuracy and precision than a classical UV-Visible assay. This technique is more cost-effective, direct, and rapid than other methods and can be used for bulk drugs and various dosage forms. This multivariate standardization method simplifies the individual result and converts it into an "m" value as a reliant variable. Within optimized conditions, this analytical technique would provide excellent sensitivity, resolving power, expeditiousness, and cost-effectiveness for a validated quantification of KETO. The absorbance of an analyte (X), i.e., KETO, is scanned at 7 different absorbances (λ = 316, 319, 322, 325, and 328nm); the following formula can then be applied for any preferred wavelength **[19-26]**.

$A_{\lambda 316} = a\ X\ C_x + \ k_1$ ---------- (1)

$A_{\lambda 319} = b\ X\ C_x + \ k_2$ ---------- (2)

$A_{\lambda 322} = c\ X\ C_x + \ k_3$ ---------- (3)

$A_{\lambda 325} = d\ X\ C_x + \ k_4$---------- (4)

$A_{\lambda 328} = e\ X\ C_x + \ k_5$---------- (5)

Where A_λ is the analyte's absorbance, a, b, c, d, and e being slopes of the analyte's linear regression functions; intercepts are denoted as k1, k2, k3, k4, k5 at the five specified wavelengths, and Cx is the analyte's concentration. The selected five equation systems (1–5) listed above can be summarised as follows:

$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + \ d\ X\ C_x + \ e\ X\ C_x + \ K_T$---------- (6)

The above equation can be further condensed to

$A_T = C_x\ (a + b + c + d + e) + \ K_T$---------- (7)

A_T and K_T are the summations of the absorbance acquired cum totality of intercepts of regression equations at selected five wavelengths, respectively. The following formula computes the concentration of the analyte X.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

MATERIALS AND METHODS

Chemicals and reagents

- Distilled water
- KETO was obtained as a gift sample from Ideal Analytical and Research Institute, Pondicherry. The marketed tablet formulation used was Cadloc, (Label claim – 10 milligram KETO) acquired from a local market.

Instrumentation

- LAB INDIA 3092 UV-Visible double beam spectrophotometer
- Ultra Sonicator Bath
- Analytical balance
- Micropipette

Analytical method development

Choice of the solvent

In water, KETO was found to be freely soluble. Hence, Water was used for further dilutions of both standard and sample.

Standard stock solution

KETO standard stock solution was prepared by dissolving 10 mg of the standard drug in 10 mL of Water and then making up to the mark in a 100 mL standard flask with the same solvent. Several concentrations (7 - 13 µg/mL) of solution were prepared from this standard stock solution.

Determination of λ_{max}

The standard stock solution was diluted in Water to obtain 10µg/mL. These solutions were measured in the Ultra-Violet region from 200 - 400 nm. The λ_{max} was obtained as 322 nm **(Figure 2)**. The linear curve was obtained with a graph plotting the absorbance against the concentration **(Table 1)**. The solutions were scanned across the range surrounding 322 nm, i.e., 316, 319, 322, 325, 328 nm, to enhance the correlation and diminish instrumental oscillations.

Preparation of sample solution

Twenty tablets of KETO were accurately weighed and powdered. A weight corresponding to 10 mg was measured into a 100 ml volumetric flask, dissolved, and

made up to the mark with KETO to obtain 100 µg/mL. This solution was then filtered and used for further analysis.

Method Validation

According to ICH Q2B guidelines, this method was validated for sensitivity, precision, accuracy, and linearity.

Linearity

The different concentrations over the range of 7 - 13 µg/mL were prepared from the standard stock solution of KETO. To minimize instrumental fluctuations and to better the correlation, these solutions were scanned over a range of wavelengths surrounding their absorbance maxima at 316, 319, 322, 325, and 328 nm, respectively. The absorbances were recorded, and the standardizations were obtained by plotting a concentration vs. absorbance graph. **(Figure 3, Table 1)**.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg/mL)	Absorbance				
	316 nm	319 nm	322nm	325 nm	328 nm
7	0.368	0.383	0.391	0.387	0.375
8	0.418	0.435	0.443	0.441	0.427
9	0.468	0.487	0.496	0.493	0.478
10	0.518	0.541	0.549	0.546	0.531
11	0.568	0.592	0.602	0.598	0.581
12	0.618	0.644	0.644	0.651	0.632
13	0.665	0.689	0.696	0.695	0.686

[#]Average of 5 determinations; UV= Ultra violet

The sensitivity of the method was determined by calculating the detection and quantification limit using the below formula.

$LOD = 3.3\ \sigma/S$ …….... (8)

$LOQ = 10\ \sigma/S$..... (9)

Here, σ is the standard deviation (SD) of the lowermost concentration and S is the slope of the standard curve.

Precision

To assess the intra-day and inter-day precision, 10µg/mL solution was scanned six times in a short interval on one day for intraday precision and six different days for inter-day precision.

Accuracy

Using the standard addition technique, the recovery study for the suggested technique was resolved at 80%, 100%, and 120%. The standard and sample stock solutions were prepared. 0.8 mL of the standard was pipetted into three 10 mL volumetric flasks. 0.8, 1, and 1.2 mL of sample solution were added, respectively, making up to a capacity of 10 mL with water. These solutions were measured with a UV spectrophotometer, and the percentage recovery was calculated.

Assay

The amount of KETO present in the tablet formulation was calculated by measuring the absorbance of the extracted tablet solution at 322 nm.

RESULTS AND DISCUSSION

The λ_{max} of KETO was found to be 322 nm with Water as the solvent as shown in **Figure 2**.

The technique is linear within the assigned concentration range of 7 - 13 µg/mL. The linear regression analysis shows an excellent linear relationship with R^2=0.9991 - 0.9999 for all the calibration plots. For precision, the % relative standard deviation was found to be 0.308 and 0.28719. The LOD and LOQ obtained are 0.011 and 0.035 µg/mL, respectively. Therefore, the values found fell according to ICH guideline limits of validation parameters.

Linearity

The linearity was recorded at 316, 319, 322, 325, and 328 nm in the concentration range of 8 - 24 µg/mL and depicted in **Figure 3**, and corresponding calibration curves are presented in **Figures 4 to 8**. For each wavelength, the low values of % relative standard deviation show that the technique is accurate and precise. The LOD and LOQ were calculated and reported in **Table 2**.

Precision

The low standard deviation values indicate that this technique is specific, and % RSD for the intra-day and inter-day precision were found to be 0.3080 and 0.2871, respectively. It lies within the limits of less than 2% at each wavelength. The low percentage value of relative standard deviation reveals that the suggested technique is accurate and precise **(Figure 9, 10)**.

Recovery

As per ICH guidelines, the % recovery of KETO was from 99.00% to 101.67% w/w. The recovery was within the acceptable range of 97 - 103 % w/w **(Figure 11, Table 3)**.

Assay:

The UV absorbance of the tablet formulation was recorded at 322 nm. The quantity and assay percentages are 9.94 mg and 99.43 % w/w, respectively, with % RSD values as in **Table 4**.

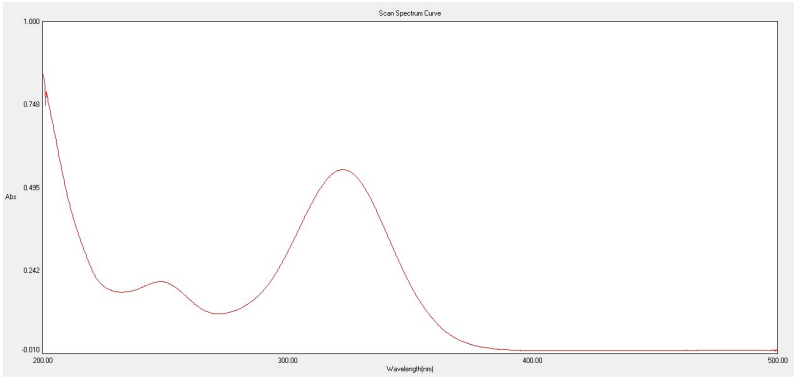

Figure 2: UV spectrum of Ketorolac (10 µg/mL), λ_{max} at 322 nm

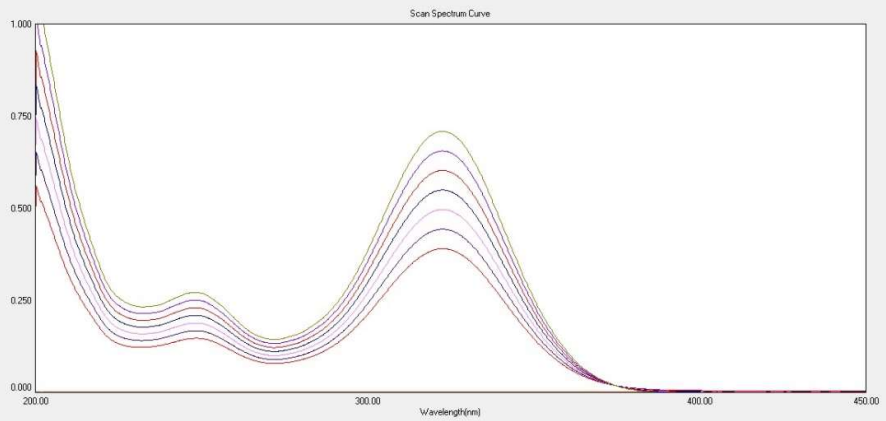

Figure 3: UV Spectrum of Ketorolac showing linearity at 322 nm

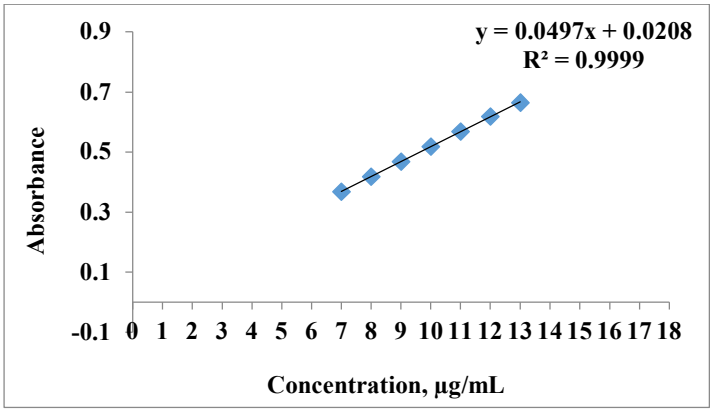

Figure 4: Calibration curve at 316 nm

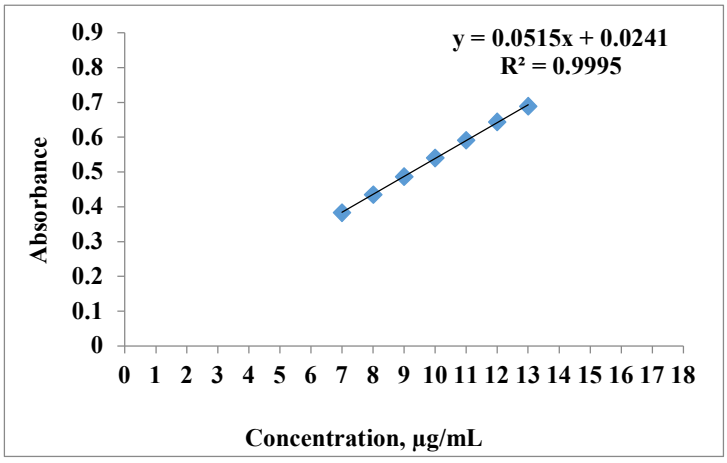

Figure 5: Calibration curve at 319 nm

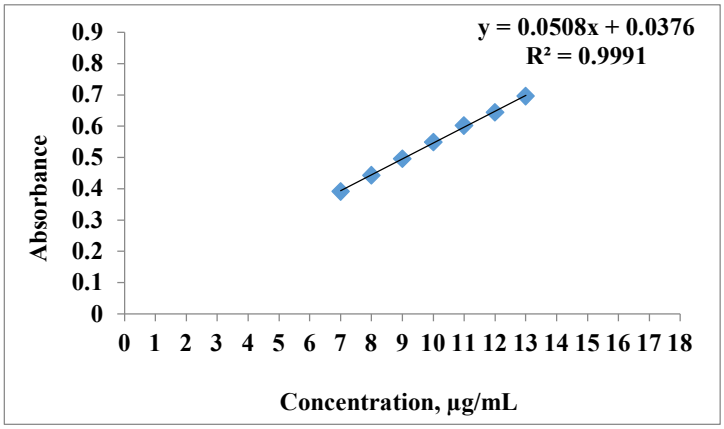

Figure 6: Calibration curve at 322 nm

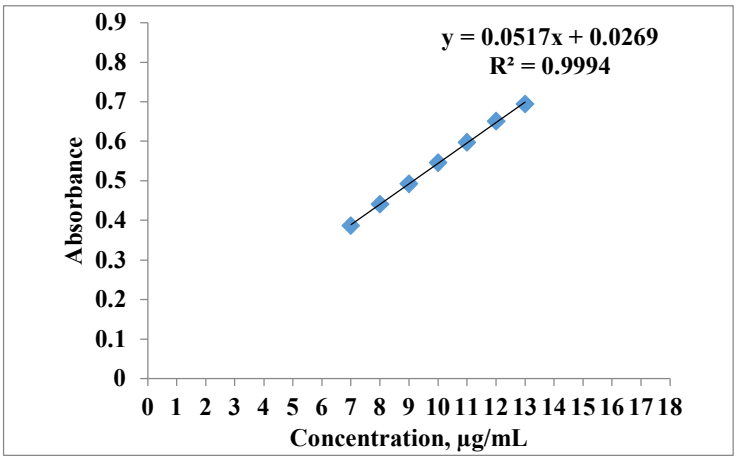

Figure 7: Calibration curve at 325 nm

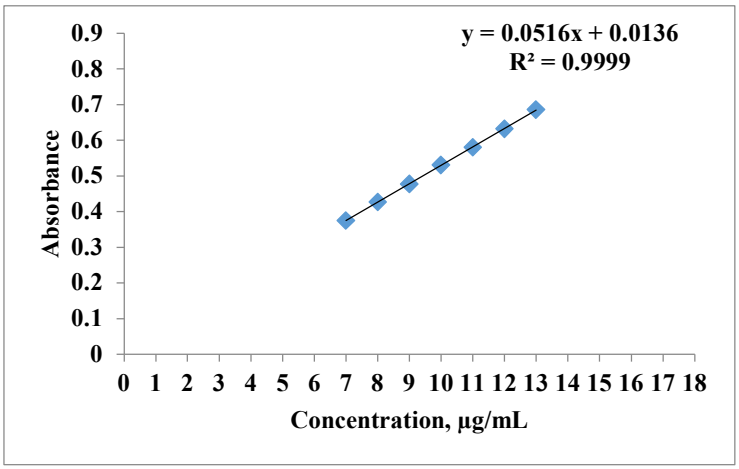

Figure 8: Calibration curve at 328 nm

Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	Regression equation	R2	LOD (µg/mL)	LOQ (µg/mL)	% RSD
316	y = 0.0497x + 0.0208	0.9999	0.0032	0.0098	0.189
319	y = 0.0515x + 0.0241	0.9995	0.0085	0.0258	0.479
322	y = 0.0508x + 0.0376	0.9991	0.0118	0.0357	0.655
325	y = 0.0517x + 0.0269	0.9994	0.0095	0.0290	0.532
328	y = 0.0516x + 0.0136	0.9999	0.0031	0.0094	0.177

*nm = nanometre; µg/mL = Microgram per millilitre

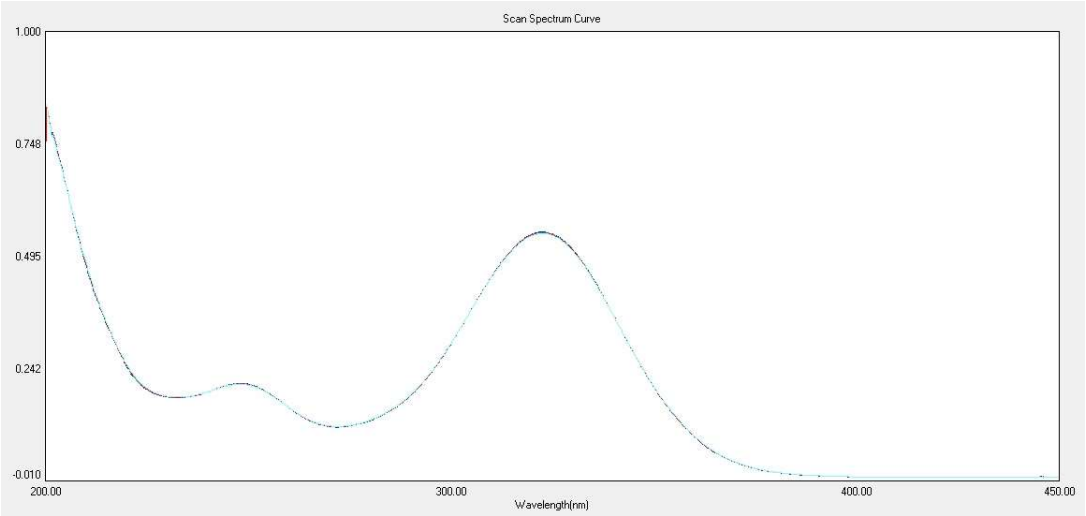

Figure 9: UV spectra showing intraday precision

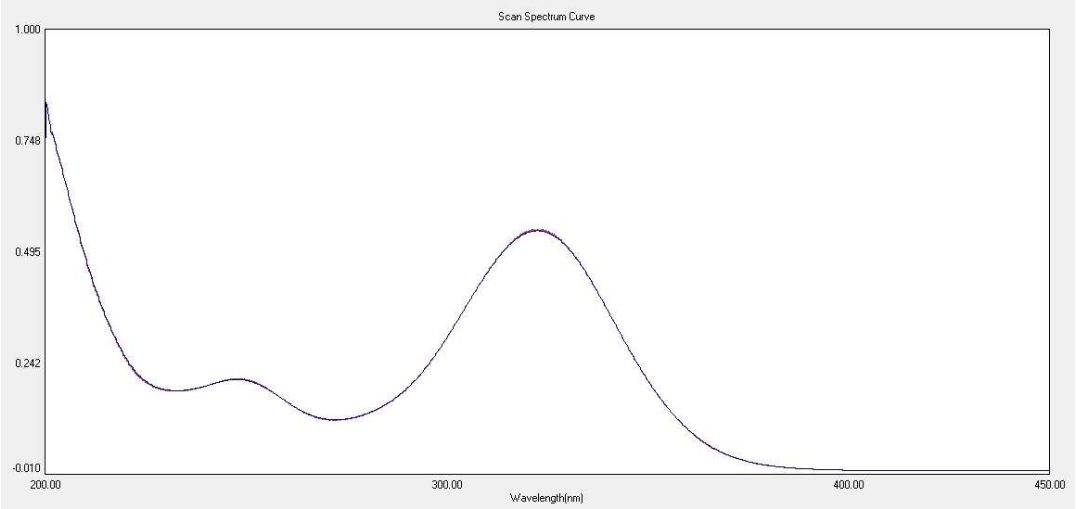

Figure 10: UV spectra showing interday precision

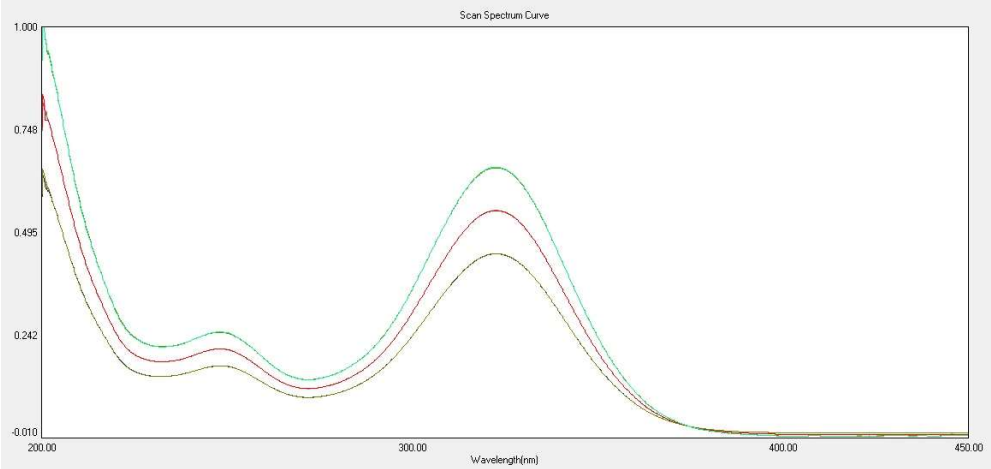

Figure 11: UV Spectrum showing accuracy of Ketorolac

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
316 nm	5	3	0.371	7.9	98.75
		5	0.451	10.1	101.00
		7	0.532	12.2	101.67
319 nm	5	3	0.421	8.1	101.25
		5	0.509	9.85	98.50
		7	0.601	11.9	99.17
322 nm	5	3	0.448	8.12	101.50
		5	0.545	9.9	99.00
		7	0.635	12.2	101.67
325 nm	5	3	0.432	8	100.00
		5	0.522	10.03	100.30
		7	0.614	12.07	100.58
328 nm	5	3	0.371	8.05	100.63
		5	0.441	10.1	101.00
		7	0.512	12.1	100.83

Table 4: Assay of Ketorolac

Label claim (mg)	Amount obtained (mg)	% Assay
10	9.91	99.10
10	9.95	99.50
10	9.97	99.70
Average	9.94	99.43
SD		0.3055
% RSD		0.3072

CONCLUSION:

This novel multivariate technique is more accurate, precise, reproducible, cost-effective, and sensitive than classical UV-Visible Spectrophotometry for KETO assay. This multilinear regression analysis is proven desirable for the testing standard drug and other dosage forms of KETO. This method is validated using ICH Quality Guidelines and found to be within the set limits of validation. This is a simple working procedure compared to expensive and intricate techniques such as HPLC and HPTLC, and hence can be employed for routine analysis of KETO in bulk drugs and pharmaceuticals.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

FUNDING SOURCES

There is no funding to report.

REFRENCES

[1] https://en.wikipedia.org/wiki/Ketorolac, Assessed 4 November 2022

[2] National Center for Biotechnology Information. PubChem Compound Summary for CID 3826, Ketorolac. https://pubchem.ncbi.nlm.nih.gov/compound/Ketorolac. Accessed 6 November 2022.

[3] Rooks WH, The analgesic and antiinflammatory profile of Ketorolac and its tromethamine salt, Drugs under Experimental and Clinical Research, 1985, 479-492.

[4] Patil VP, Devdhe SJ, Angadi SS, Kale SH, Phalke SD, Shelke SD, Patil RH. Validated spectrophotometric method for the estimation of Ketorolac tromethamine in bulk and tablets using Ninhydrin- A modified approach. Asian J. Res. Chem. 2014; 7(1): 1-24.

[5] Atkoşar Z, Altiokka G, Ergun B, Genç L. The determination of ketorolac tromethamine in pharmaceutical preparations by flow injection analysis using UV detection. Acta Pharm. Turc. 2001; 43(2): 143-146.

[6] Chaudhary J, Akash J. Simultaneous estimation of multicomponent formulations by UV - Visible Spectroscopy: An Overview. Int. Res. J. Pharm. 2011; 2(12): 81-83.

[7] Shukla T, Pandey SP, Upmanyu N. Simultaneous estimation of Thiocolchicoside and ketorolac tromethamine using UV spectroscopy. In J. Indian Chem. Soc. 2019; 96: 305-310.

[8] Gayatri AP, Srinivas K, Nageswara Rao K, UV Spectrophotometric and stability indicating RP-HPLC methods for simultaneous estimation of Moxifloxacin HCl and Ketorolac tromethamine in bulk and ophthalmic dosage forms. Asian J. Res. Chem., 2022. https://doi.org/10.52711/0974-4150.2022.00006.

[9] Kaur R, Sukhdev Singh AH, Jalhan S, Jain UK. Development and validation of UV Spectrophotometric method for the estimation of Ketorolac Tromethamine in bulk drug. World J. Pharm. Pharm. Sci. 2012; 5(4): 1-4.

[10] Harsha DS, Sushma, G, Annapurna MM. First derivative spectrophotometric methods for the determination of Ketorolac Tromethamine tablets. Acta Sci. Pharm. Sci. 2020; 4(5): 1-5.

[11] Kumbhar D. Experimental design approach for chromatographic determination of Ketorolac tromethamine from bulk drug and tablet formulation. Glob J Pharm Sci, 2017;3(2):1-10.

https://doi.org/10.19080/gjpps.2017.03.555609.

[12] Connor N, Geary M, Wharton M, Curtin L. Development and validation of a rapid liquid chromatographic method for the analysis of ketorolac tromethamine and its related production impurities. J. Appl. Pharm. Sci. 2012; 2(5): 1-5. https://doi.org/10.7324/JAPS.2012.2524.

[13] Ganta S, Vidyadhara S, Srilakshmi , Ganji R, Kavati R. Development and validation of a stability indicating RP-HPLC method for the estimation of Ketorolac tromethamine in drug substance and its pharmaceutical formulations. Indo Am. J. Pharm. Sci. 2014; 80-89. http://www.iajps.com.

[14] Pudi R, Annapurna MM. Method development and validation of a new stability-indicating liquid chromatographic method for the quantification of ketorolac tromethamine a nonsteroidal anti-inflammatory drug. Asian J. Pharm. 2012; 12: 674-678.

[15] Uddin MN, Al-Amin M, Mijan Md NH, Das S. Degradation study of Ketorolac tromethamine by a validated UPLC method, Indonesian Journal of Pharmaceutical Science and Technology, 2018;6(1):11-26. https://doi.org/10.24198/ijpst.v6i1.16508.

[16] Tambe VS, Deodhar MN, Prakya V. LC and LC–MS study for simultaneous determination of tramadol hydrochloride and ketorolac tromethamine in bulk and formulation with their major degradation products. Bull. Fac. Pharm. (Cairo Univ.), 2016;54(1):1-5. https://doi.org/10.1016/j.bfopcu.2016.02.002.

[17] Devarajan PV, Gore SP, Chavan SV. HPTLC determination of ketorolac tromethamine. J. Pharm. Biomed. Anal. 2000; (22): 679-683.

[18] Rao PKLM, Venugopal V, Teja Ch SG, Radhika DVNS, Kavitha K, Lavanya S. Revalidation and analytical evaluation of Ketorolac tromethamine by HPTLC using reflectance scanning densitometry. Int. J. Res. Pharm. Chem. 2011; (2): 129-132.

[19] Kokilambigai KS, Seetharaman R, Kavitha J, Sowndaravel P, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification

of Zaleplon in bulk and pharmaceutical formulations. J. Pharm. Sci. Res. 2017; 9(6): 824-9.

[20] Saisusmitha A, Kokilambigai KS, Lakshmi KS. Spectrophotometric quantification of Telmisartan employing multivariate calibration technique in bulk and pharmaceutical formulations. Res. J. Pharm. Technol. 2019; 12(4): 1799-805. DOI: 10.5958/0974-360X.2019.00300.7.

[21] Madhan S, Kavitha J, Lakshmi KS, 2016. Multivariate calibration technique for the spectrophotometric quantification of Ivermectin in pharmaceutical formulation. Asian J. Pharm. Clin. Res. 2019;12(2): 444-51.

[22] Durgadevi P, Kokilambigai KS, Lakshmi KS. First order derivative spectrophotometric method for the quantification of Telmisartan employing multivariate calibration technique. Res. J. Pharm. Technol. 2020;13(2):774-80. DOI: 10.5958/0974-360X.2020.00146.8.

[23] Naveena Rani D, Kokilambigai KS, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of Rasagiline in bulk drug and pharmaceutical formulation. Res. J. Pharm. Technol. 2020;13(2):843-9. DOI: 10.5958/0974-360X.2020.00159.6.

[24] Mani Aravinth R, Kokilambigai KS, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi KS. Spectrophotometric quantification of Granisetron in bulk drug and pharmaceutical formulations employing multivariate calibration technique. J. Med. Pharm. Allied Sci. 2021;10(4):3435-9. DOI: 10.22270/Jmpas.V10I4.1442.

[25] Vimal Ravi, Kokilambigai KS, Kavitha J, Seetharaman R, Lakshmi KS. 2022. Multivariate UV spectrophotometric quantification of Cilnidipine in bulk drug and pharmaceutical formulations. J. Med. Pharm. Allied Sci. 2022; 11(2): 4672- 8

[26] Kokilambigai KS, Logesh M, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi KS. Multivariate calibration technique for spectrophotometric quantification of Ondansetron in bulk drug and pharmaceutical formulations. 2022; 11(4): 4777-82.