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ANALYSIS OF KETOROLAC IN HUMAN URINE AND SIMULATED HUMAN URINE BY UHPLC-DAD: METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT

Ketorolac is a member of the pyrrolo-pyrrole group of non-steroidal anti-inflammatory drug. It is a potent antipyretic and anti-inflammatory. It is mainly used for the short term treatment of post-operative mild to moderately severe pain by inhibiting the bodily synthesis of prostaglandins. In the present study, new UHPLC-DAD method for the analysis of KTR in standard form, spiked simulated urine and spiked human urine samples was developed and validated in the present investigation. The method utilized C18 as stationary phase and a combination of acetonitrile: water pH 4(3:7) as an eluent. It was found that selected eluent provided short run time (2.4 min.), and better peak symmetry. Excellent linear relationship (r^2 0.9996) was observed for linear regression data for the calibration plots. The developed system was validated according to ICH guidelines. Analysis of KTR in spiked simulated urine and spiked human urine samples gave recovery of 99.47 % and 101.51% of drug respectively. Results obtained from the statistical treatment of the values obtained for different parameters proved that the method is suitable, reproducible and selective for the analysis of KTR in bulk, spiked simulated urine and spiked human urine samples.

KEYWORDS: Ketorolac, UHPLC-DAD, Chromatography, Simulated Urine, Urine.

1. INTRODUCTION

The analysis of drugs in biological fluids provides critical information necessary to evaluate

the safety, efficacy and mechanism of action of pharmaceuticals.[1] Ketorolac tromethamine is chemically (4-)-5-benzoyl- 1,2-dihydro-3H-pyrrolo[1,2-a]pyrrole-1-carboxylic acid 2-amino-2-(hydroxymethyl)-l,3-propanediol (Fig. 1).

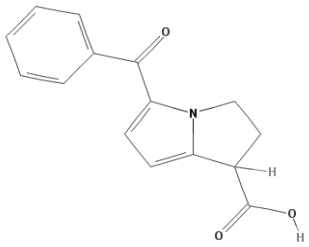

Fig. 1: Structure of Ketorolac.

It is a member of the heterocyclic acetic acid derivative family having a highly potent analgesic and anti-inflammatory activity similar to that of the opioids.[2-3] It is efficacious in treating pain arising from a broad spectrum of causes, such as postpartum and postoperative pain, cancer pain, and pain from dental extraction.[4] The drug is administered intravenously, intramuscularly, or orally as the water soluble tromethamine salt (Toradol; Hoffmann-La Roche, USA) to treat moderate pain with reduced opioid doses.

There are a number of methods (spectrophotometric, fluorimetric assay, HPLC, HPTLC, and capillary electrochromatography) described in the literature for the analysis of ketorolac in biological samples such as in plasma.[5- 12] and serum.[13-14] Some earlier investigators used various sample clean-up procedures involving organic solvent extraction of serum or plasma samples.[15] Another group reported method involving extraction with ethyl acetate hexane (30:70, v/v) after acidification.[16] For the simultaneous determination of ketorolac and p-hydroxyketorolac a three-step extraction procedure was reported[17], but the amount of sample handling was great. A HPLC/DAD-FLD method combined with prior Electromembrane extraction (EME) was developed for the determination of six widely used nonsteroidal anti-inflammatory drugs (NSAIDs): salicylic acid (SAC), ketorolac (KTR), ketoprofen (KTP), naproxen (NAX), diclofenac (DIC) and ibuprofen (IBU) in urban wastewaters.[18] In a report[19] authors describe the direct extraction of trace levels of a neutral (flavone) and an acidic (ketorolac) drug from a plasma matrix using supercritical CO_2. In yet another report, a batched solid-phase extraction method for the isolation of ketorolac from postmortem blood; liquid chromatography with multiple wavelength photodiode array detection is used for the

analysis. Confirmatory analysis was performed by gas chromatography-mass spectrometry (GC-MS) following derivatization.[20]

There has been much emphasis recently, on reducing analysis time and solvent consumption in HPLC. The advances in column technology and the introduction of a number of commercially available ultra-high performance liquid (UHPLC) chromatographs, has permitted this reduction. Ultra-high performance liquid chromatography (UHPLC) is a relatively new and advanced liquid chromatographic technique which makes the resolution possible in significantly less time because of very fine particle size columns (~3µm) with significantly lesser consumption of eluents.[21]

All the above cited reports deals with the determination of KTR either in plasma or serum but none of them discussed their determination in human urine. Therefore, the aim of the present study was to develop and validate a simple, cost effective, rapid, facile, selective, precise, reproducible, accurate, robust and stability-indicating RP-UHPLC method coupled with UV detection for the rapid analysis of KTR in bulk drug, marketed formulation, simulated urine and human urine samples utilizing acetonitrile: 0.1% aq. acetic acid (pH 4) (3:7) as mobile phase taking into consideration a variety of international conference on harmonization (ICH) recommended test conditions (ICH, 2003).

2. MATERIALS AND METHOD

2.1 Chemicals and reagents

Ketorolac (purity 99.9%) was purchased from Riyadh Pharma, (Riyadh, Saudi Arabia). HPLC grade acetonitrile was procured from BDH Laboratory supplies (Liverpool, UK). All other reagents and chemicals employed were of analytical reagent (AR) quality.

2.2 Instrumentation and chromatographic conditions

Analysis of KTR was done at room condition (22 ± 1 °C), with Thermo Scientific UHPLC system (Thermo Scientific, Germany) provided with a 3000 LC pump, 3000 autosampler, binary pumps, a programmable DAD detector, ultimate 3000 column oven, ultimate 3000 controller and an inline vacuum degasser and based on Chromeleon software, version 6.8. Chromatography was performed on a Thermo Hypersil GOLD 50 × 2.1 mm reversed phase C18 column (Thermo Scientific, Germany) having a 1.9 µm size particle as a static phase. The mobile phase consisted of acetonitrile: 0.1% aq. acetic acid (pH 4) (3:7). The

chromatography was done at a flow rate of 0.5 mL min^{-1} with DAD detection at 320 nm. Samples (10 µL) were introduced using an ultimate 3000 series Thermo auto sampler.

2.3 Preparation of KTR stock solution

Linearity plot for KTR was plotted in concentration range of 0.05 to 5µg mL^{-1}. Working dilutions in the desired range were prepared from the stock solution (100 µg mL^{-1}) by suitably mixing the required aliquots with the mobile phase to get the desired concentration.

2.4 Method development

Various mixed organic/hydro-organic solvent systems as mobile phase were tried for the development of suitable UHPLC–DAD method for the simultaneous quantification of KTR in its standard drug compound. Various parameters were kept in mind while deciding the suitability of any solvent or mixture of solvents to be used as mobile phase such as appropriatness for stability studies, sensitivity of the developed method, time consumed for the analysis, peak parameters, mutual miscibility of the constituent solvents and the economy of the solvents. Based upon above criterions, various mobile phases such as methanol–water, methanol–phosphate buffer, acetonitrile–phosphate buffer, methanol–sodium percholate buffer and acetonitrile–sodium percholate buffer in varying proportions were tried. Among the various tried solvents systems for UHPLC quantification, a combination of acetonitrile (30): 0.1% acetic a in water PH 4 (70) was selected as most suitable eluent for subsequent studies.

2.5 Validation studies

The newly developed UHPLC–DAD method was validated for various parameters such as linearity, precision, accuracy and stability.[22] Freshly made dilutions in the concentration range of 0.05–05 µg mL^{-1} were used for plotting of linearity curves. The solvent system consisting of acetonitrile (30) :0.1% acetic a in water PH 4 (70) was dispatched at 0.5 mL min^{-1} for bringing column to equilibrium and the baseline was continuously observed and monitored during the entire process. The drug KTR was detected at 320 nm. The freshly prepared dilute solutions were introduced into the system in the multiples of three and peak areas were recorded using UHPLC system for each solution and calibration was obtained by plotting concentration vs. peak area.

Precision of the newly developed UHPLC–DAD method was estimated at two different levels i.e. repeatability (intraday precision) and interday (intermediate) precision. Intraday

precision of the proposed UHPLC–DAD method was carried out by quantification of five different concentrations of KTR solution (0.1, 0.5, 1, 3, and 5 μg mL^{-1}) three times on the same day. However, intermediate precision of the proposed UHPLC–DAD method was determined by reanalyzing the samples on three different days.

2.6 Application of UHPLC–DAD method for the assay KTR in simulated urine as well as human urine.

The validity of UHPLC–DAD method was identified by applying it for the assay of KTR in simulated urine as well as human urine.

Condition of extration of KTR from urine

2 ml drug solution (01 μg/ml conc.) + 2 ml (urine) + 1 ml (0.5N HCl) were poured in a separating funnel with 10 ml chloroform and shaken for 5 min. and then left for 5 min for separation of aqueous phase from organic phase. Subsequently, 9 ml solution was collected from chloroform layer and 09 ml chloroform was added to the separating funnel and shaken for 5 min. Then separating funnel was put to rest for 5 min in order to separate aqueous layer from organic layer. 9 ml solution from chloroform layer was again taken. The net 18 ml solution from chloroform layer (collected in two steps) was applied to rotavapor for evaporating chloroform. The solid content from the chloroform layer was dissloved in 10 ml triethanolamine and used as such for analysis.

Preparation of simulated urine

The following reagents will be necessary for the preparation of normal human urine. A primer of urinalysis (2nd ed.). New York: Hoeber Medical Division, Harper & Row, Publishers.): Distilled water, Potassium chloride (0.38 g), Sodium chloride (13.75 g), calcium chloride (0.665 g), magnesium sulphate (01.69 g), magnesium chloride (0.825 g) and urea (17.3 g). Simulated urine was prepared by following the earlier reported protocol (Kark, R.M., Lawrence, J.R, Pollack. V.E., Pirani. CL., Muehrcke, RC. & Silva, H. (1964). In short, initially 24.26 g of urea was mixed in double distilled water. When urea crystals got completely dissolved in water, 10 g of sodium chloride, 6.0 g of potassium chloride and 6.4 g of sodium phosphate were also added to urea solution and shaken till the solution becomes clear. pH and specific gravity of this solution was tested and found to be in the range 6.4 (pH range for normal urine) and 1.012 (specific gravity range 1.015 - 1.025 for normal urine). The prepared solution was kept in refrigerator and was used as stock solution of simulated urine solution. The stock simulated urine solution was slightly warmed to room temperature before

use. The simulated urine solution was spiked with KTR solution equivalent to 01 µg/ml concentration and then analysed for the recovery of amount of KTR that can be analysed with this developed UHPLC method.

3. RESULTS AND DISCUSSION

3.1 Chromatography and Method validation

The important criteria for development of successful UHPLC method for analysis of KTR in bulk drug, simulated urine and urine were: the method is expected to be able to resolve KTR satisfactorily and should also be accurate, reproducible and robust. It should also be able to analyse drug in simulated urine/ urine and simple enough for routine use in quality control laboratory.

During method development step, use of methanol and phosphate buffer (pH 7, pH 4, pH 8) was monobasic potassium phosphate and sodium hydroxide) as the mobile phase produced asymmetric peak with a larger tailing factor (> 2) and fewer number of theoretical plates (< 2000). Further, acetonitrile was tried in combination of phosphate buffer at different proportions at flow rate of 0.5 mL/min. A chromatogram was obtained with unsatisfactory peak parameters when pH of the buffer was either 7 or 8 but in case of pH 4, a slightly better peak was obtained. In yet another attempt to get a satisfactory peak having asymmetry factor < 2 and good sensitivity, acetonitrile and water (pH adjusted by acetic acid) as another eluting phase tried. Various compositions of acetonitrile and water (pH 4) were tested, the binary proportion at 30: 70% v/v was found superior with a sharp peak, appropriate retention time and fine asymmetry. Thus, proportion of the acetonitrile and water (pH 4) at 30: 70 was used to develop a facile and quick method for KTR with a reasonable run time (2.40 min), appropriate retention time 1.877 ± 0.01 min and the acceptable tailing or asymmetry factor [Figure 2]. The resolution of KTR was studied using this mixture of acetonitrile and water (pH 4) at 30: 70 on UHPLC C_{18} column and system.

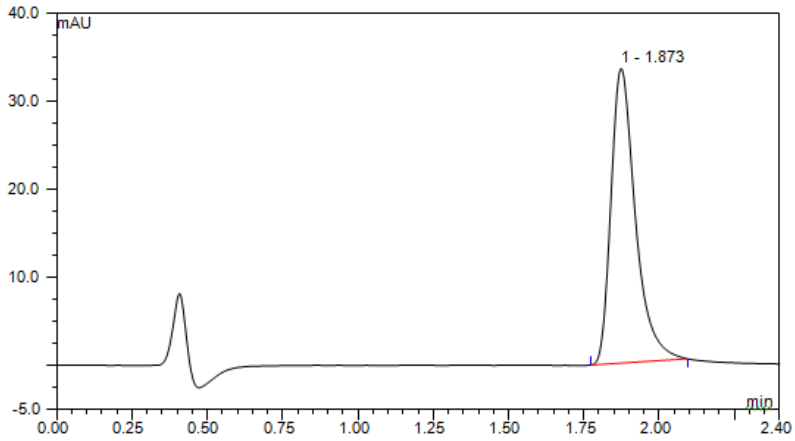

Fig.2: RP-UHPLC chromatogram of KTR in acetonitrile: water (pH 4) at 30: 70 % v/v) with retention time of 1.873 min.

3.2 Validation of the Method

3.3.1 Linearity

The linearity of detector response to different concentrations of drugs was studied in the range of 0.05–5 µg mL^{-1} at 8 different concentrations (0.05, 0.1, 0.3, 0.5, 1, 1.5, 2, 3, 4 and 5 µg mL^{-1}). The samples were analyzed in triplicates at all concentrations. Calibration curves were constructed and found that correlation coefficient value of the drug was observed to be 0.999. The regression analysis data for calibration curves were calculated using the peak areas and the data are shown in Table 1.

3.3.2 Precision

The intraday precision (repeatability) of sample was evaluated as intraday variation whereas the intermediate precision was evaluated by measuring inter-day variation for analysis of KTR at five different concentrations (0.1, 0.5, 1, 3, and 5 µg mL^{-1}), in triplicate. Data obtained from determination of repeatability and intermediate precision expressed as % RSD were shown in Table 3 & 4. The low magnitude of % RSD indicates the high repeatability of the method.

3.4 Application of developed method for the analysis of FNF and SMV in developed SEDDS

The developed RP-UHPLC method was found to be quick, sensitive enough and suitable for the quantitative estimation of KTR. Therefore, this method was put to test for the estimation of KTR in spiked urine and spiked simulated urine samples. The amount of KTR in spiked urine and spiked simulated urine samples was found to be 99.47 % and 101.517%,

respectively. High assay value and low % RSD (0.3654 % for spiked urine and 0.1425% for spiked simulated urine samples respectively of KTR indicated that the method is suitable for analysis of KTR in bulk drug and biological samples. The chromatogram of KTR analysis extracted from spiked urine and spiked simulated urine samples was matching with that of standard KTR, showing the purity of peak in tested matrices. (Figure 3)

Tables with captions

Table 1: Linear regression data for calibration plots for SMV and FNF (n = 3).

Parameters	Values
Linearity range (ng per spot)	0.05 - 100 µg/mL
Regression equation	y = 0.6164x + 0.0.0097
Correlation coefficient	0.9996
Slope ± SD	0.6224 ± 0.0054
Confidence interval of Slope	0.013435
Intercept ± SD	0.0094± 0.0002
Slope without intercept ± SD	0.6193
Standard error of slope	0.0031
Standard error of intercept	0.0001
Confidence interval of intercept	0.0006

Table 2: Selectivity of proposed HPLC method (n = 6).

Conc. (µg/mL)	Peak Area	Mean Area ± SD	RSD (%)	Rt (min)	Mean Rt ± SD	RSD (%)
2	1.2462	1.2508 ± 0.0071	0.5680	1.877	1.8728 ± 0.0026	0.1406
	1.2485			1.873		
	1.2437			1.870		
	1.2477			1.874		
	1.2622			1.870		
	1.2568			1.873		

Table 3: Precision of proposed HPLC method (n = 3).

Conc. (µg/mL)	Repeatability (Intra-day precision)			Intermediate precision (Inter-day)		
	Mean area ± SD	RSD (%)	Standard error	Mean area ± SD	RSD (%)	Standard error
0.1	0.0646 ± 0.0020	3.1306	0.0012	0.0656 ± 0.0008	1.3549	0.0005
0.5	0.194133 ± 0.0047	2.4272	0.0027	0.1961 ± 0.0010	0.5109	0.0006
1	0.6485 ± 0.0046	0.7113	0.0026	0.6512 ± 0.0032	0.4968	0.0018
3	1.927367 ± 0.0166	0.8602	0.0096	1.9441 ± 0.0073	0.37692	0.00423
5	3.1856 ± 0.0168	0.5292	0.0097	3.2083 ± 0.0134	0.4164	0.0077

Table 4: Analysis of KTR in spiked urine and spiked simulated urine samples.

Sample	Amount of spiked drug (µg mL^{-1})	% Recovery ± SD	RSD (%)
Spiked urine	01	99.47 ± 0.0036	0.3654
Spiked simulated urine	01	101.51 ± 0.0014	0.1425

4. CONCLUSION

It can be concluded from the present study that acetonitrile and water (pH 4) at 30: 70 ratio can be employed as an eluent for the analysis of KTR. The developed method was found to be linear over a wide concentration range as well as exhibiting good precision, accuracy and recovery properties. The newly developed was found applicable for the analysis of KTR in spiked urine and spiked simulated urine samples indicating its suitability for the routine analysis of of KTR in different biological matrices.

5. Conflict of Interest

The author report no conflict of interest related with this manuscript.

6. ACKNOWLEDGEMENT

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7. REFERENCES

1. A. Yacobi, J.P. Skeily and V.K. Batra, Toxicokinetics and New Drug Development, Pergamon Press, New York, 1989.
2. E. J. Mroszczak, F. W. Lee, D. Combs, E. H. Sarnquist, B. Huang, A. T. Wu, L. G. Tokes, M. L. Maddox and D. K. Cho, Drug Metab. Dispos, 1987; 15: 618.
3. Gillis JC, Brogden RN. Ketorolac. A reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic use in pain management. Drugs, 1997; 53: 139-188.
4. B. J. Floy, C. G. Roylo and J. S. Fleitman, Am. J. Hosp. Pharm, 1990; 47: 1097.
5. Devarajan P, Gore S and Chavan S, HPTLC determination of ketorolac tromethamine, J. Pharmaceutical and Biomedical Analysis, 2000; 22(4): 679-683.
6. Tsina I, Tam Y, Boyd A, Rocha C, Massey I and Tarnowski T, An indirect (derivatization) and a direct HPLC method for the determination of the enantiomers of ketorolac in plasma, J. Pharmaceutical and Biomedical Analysis, 1996; 15(3): 403-417.
7. Tsvetkova BG, Pencheva IP, Peikov PT. HPLC determination of ketorolac tromethamine in tablet dosage forms. Der Pharmacia Sinica, 2012; 3: 400e403.
8. Eid M, El-Brashy A, Aly F, Talaat W. Spectrofluorometric determination of ketorolac tromethamine via its oxidation with cerium (IV) in pharmaceutical preparations and biological fluids. J AOAC Int, 2007; 90: 941e947.

9. Razzaq SN, Mariam I, Khan IU, Ashfaq M. Development and validation of liquid chromatographic method for gatifloxacin and ketorolac tromethamine in combined dosage form. J Liq Chrom Rel Tech, 2012; 35: 651.
10. Orlandini S, Fanali S, Furlanetto S, Marras AM, Pinzauti S. Micellar electrokinetic chromatography for the simultaneous determination of ketorolac tromethamine and its impurities. Multivariate optimization and validation. J Chromatogr A, 2004; 1032: 253-263.
11. Orlandini S, Furlanetto S, Pinzauti S, D'Orazio G andFanali S, Analysis of ketorolac and its related impurities by capillary electrochromatography, J. Chromatography A, 2004; 1044(1-2): 295-303.
12. Sturm JC, Canelo H, Nunez-Vergara LJ, Squella JA. Voltammetric study of ketorolac and its differential pulse polarographic determination in pharmaceuticals. Talanta, 1997; 44: 931e937.
13. Chaudhary R, Gangwal S, Jindal K and Khanna S, Reversed phase high-performance liquid chromatography of ketorolac and its application to bioequivalence studies in human serum, J. Chromatography B: Biomedical Sciences and Applications, 1993; 614(1): 180- 184.
14. Determination of Ketorolac in Human Serum by High-performance Liquid Chromatography, In Koo Chun, Hyun Hee Kang and Hye Sun Gwak, Arch. Pharm. Res, 1996; 19(6): 529-534.
15. F. Jamali, F. M. Pasutto and C. Lemko, J. Liq. ChromatoL'r, 1989; 12: 1835.
16. N. S. Jallad, D. C. Garg, J. J. Martinez~ E. J. Mroszczak and D. J. Weidler, J. Clin. Pharmaeol, 1990; 30: 76
17. A. T. Wu and 1. J. Massey, J. Chromatogr, 1990; 534: 241.
18. Electromembrane extraction (EME) and HPLC determination of non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater samples, Maria Ramos Payan, Miguel Angel Bello Lopez∗, Rut Fernandez Torres, Mercedes Villar Navarro, Manuel Callejon Mochon, Talanta, 2011; 85: 394–399.
19. Journal of Chromatography B, 1994; 657: 206-213. Supercritical fluid extraction as a sample preparation technique for the direct isolation of drugs from plasma prior to analysis, Hanjiu Liu, Kenneth R. Wehmeyer.
20. Barry K, Patrick N Friel, Kristina L Peterson, David B. Predmore, Analysis of Ketorolac in Postmortem Blood, Journal of Analytical Toxicology, March/April 1995; 19: 61-64.

21. Wren SA, Tchelitcheff P. Use of Ultra-Performance Liquid Chromatography in Pharmaceutical Development. J Chromatogr A, 2006; 1119: 140-146.
22. Haq N, Shakeel F, Ali M, Elbadry M, Alanazi F K, Alsarra IA. An environmentally benign approach for rapid analysis of indomethacin using a stability indicating RP-HPLC method. J Liq Chromatogr Rel Technol, 2014; 37: 878-892.