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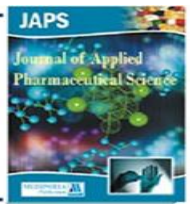

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Development and validation of a rapid liquid chromatographic method for the analysis of Ketorolac Tromethamine and its related production impurities

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ABSTRACT

A high performance liquid chromatographic (HPLC) method for the analysis of ketorolac tromethamine and its associated impurities was examined with the aim of reducing analysis times while maintaining good efficiency. The separation was carried out using a waters X-bridge – C8 (3mm x75mm, 2.5µm particle size) column with a mobile phase of tetrahydrofuran – ammonium dihydrogen phosphate (0.05M, pH3, 28:72 v/v) at a flow rate of 1.7mL/min and UV detection at 313nm. The method was validated according to ICH (international conference on harmonisation) guidelines with respect to precision, accuracy, linearity, specificity, robustness and limits of detection and quantification. All parameters examined were found to be well within the stated guidelines. Naturally aged samples were also tested to determine sample stability. A profile of sample and impurity breakdown was presented. A three-fold reduction in analysis time was achieved in comparison with the current approved EP (european pharmacopeia) method and the method can be immediately used for routine assay and related substance determination.

Keywords: Ketorolac tromethamine, active pharmaceutical ingredient, method development, validation, sub 2 µm column.

INTRODUCTION

Ketorolac trometamol, 2-Amino-2-(hydroxymethyl) propane-1, 3-diol (1RS) -5-benzyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylate (API) (European Pharmacopoeia 2008) (Fig..1A) is a non-steroidal anti-inflammatory drug (NSAID). It is a member of the heterocyclic acetic acid derivative family and is used as an analgesic with an efficacy close to that of the opioid family (Rang *et al.*, 2007). It is also a potent antipyretic and anti-inflammatory. It is mainly used for the short term treatment of post-operative pain as it is highly selective for the COX-1 enzyme (Wang *et al.*, 2001). Treatment is begun with an injection and oral use is only permitted when the patient is able to eat and drink. Its main impurities are (±) -6-Benzoyl – 1,2-dihydro-1H-pyrrolizine-1-carboxylic acid (Impurity 1) (Fig.1b), (±) -5-Benzoyl – 2,3-dihydro-1-hydroxy-1H-pyrrolizine-1 (Impurity 2) (Fig.1c) and 5-Benzoyl – 2,3-dihydro-1H-pyrrolizine-1-one, (±) -5-Benzoyl – 2,3-dihydro-1-hydroxy-1H-pyrrolizine-1 (Impurity 3) (Fig.1d).

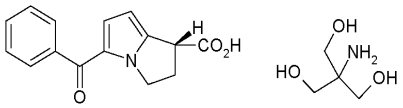

Fig. 1a: Structural formulae for ketorolac tromethamine (MW=376.4).

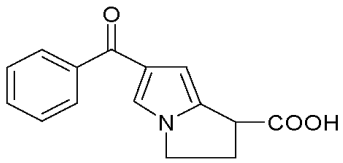

Fig. 1b: Structural formulae For Inpurity 1.

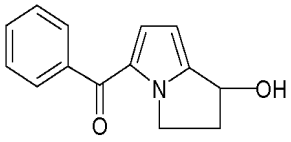

Fig. 1c: Structural formulae for impurity 2.

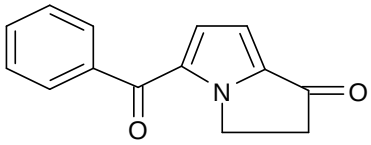

Fig. 1d: Structural formulae for Impurity 3.

There are a number of methods described in the literature for the analysis of ketorolac in biological samples, such as plasma (Tsina *et al.,* 1996) and serum (Chaudhary *et al.,* 1993), by HPLC (Jones *et al.,* 1994) (Wu *et al.,* 1990) (Ing-Lorenzini *et al.* 2009) as well as the use of other analytical chromatographic techniques such as TLC (Devarajan *et al.,* 2000) and capillary electrochromatography (Orlandini *et al.,* 2004). These are often complicated and time-consuming methods or cannot be used for the simultaneous determination of the API and its impurities.

High performance liquid chromatography (HPLC) is widely known to be one of the most important analytical techniques used in the pharmaceutical industry. Many monographs in the european pharmacopeia (Ph.Eur) report the use of chromatographic methods for the analysis of pharmaceutical substances. 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 (UPLC) chromatographs, has permitted this reduction. Many reports have shown the advantages of using these smaller particle shorter columns (Dongre *et al.,* 2008), including the reduction in analysis times while maintaining or increasing resolution, and the sensitivity and selectivity have been increased (De Villiers *et al.,* 2006) (Wren *et al.,* 2006) (Guillarme *et al.,* 2006). This improvement in speed and sensitivity is useful in all areas of the pharmaceutical process.

The aim of this research was to achieve a faster separation of ketorolac tromethamine and three main impurities in the bulk substance. An isocratic method was developed and validated according to ICH guidelines.

MATERIALS AND METHODS

Materials and reagents

Samples of ketorolac tromethamine and three impurities were received from Roche Pharmaceutical Inc., Clarecastle, Ireland. HPLC-grade tetrahydrofuran (THF) was purchased from Sigma Aldrich. Reagent-grade ammonium dihydrogen phosphate was obtained from BDH laboratories. Ultra-pure water was obtained by Millipore milli Q water purification system.

Instrumentation

The system used was an Agilent 1200 rapid resolution liquid chromatograph with a 1200 series binary pump SL and vacuum degasser, a 1200 series high-performance auto-sampler, a 1200 series thermostatted column compartment SL, a 1200 Series DAD SL for up to 80 Hz operation which were controlled by chemstation B.02.01.SR1 data acquisition and evaluation software. The column used was a waters X-bridge C8 column (75mm x 3mm i.d., 2.5µm particle size). Evaluation of an Agilent, Zorbax SB-C8, 1.8 µm column was also conduction.

Chromatographic conditions

An isocratic separation was carried out using a mobile phase consisting of THF- ammonium dihydrogen phosphate (pH3 0.05M) (28:72 v/v) was used at a flow rate of 1.7ml/min with UV detection at 313nm. The column was heated to 44°C and an injection volume of 3µl was used. The mobile phase was filtered through 0.45µm nylon filters and degassed in an ultrasonic bath prior to use.

Sample preparation

A standard solution (40µg/ml) of the ketorolac tromethamine and the three impurities was prepared using THF-Ultrapure water (28:72 v/v) as the diluents.

A high range linearity standard solutions (7 standards), containing only the API, were prepared from an 1000µg/mL stock solution over a range of 200-800µg/mL.

A low range solutions (7 standards), containing only the impurities, were prepared from a 2µg/mL stock solution over a range of 0.22 – 1.54µg/mL.

RESULTS AND DISCUSSION

Method development and optimisation

Initially, as detailed in the Ph. Eur, a waters spherisorb C8 column (250mm x 4.6mm i.d., 5µm particle size) maintained at 40°C was used for the determination of ketorolac tromethamine and its impurities. The flow rate of 1.5mL/min eluted the active ingredient in 9.12mins. After a review of the columns available, a waters X-bridge 2.5 µm column was decided upon.

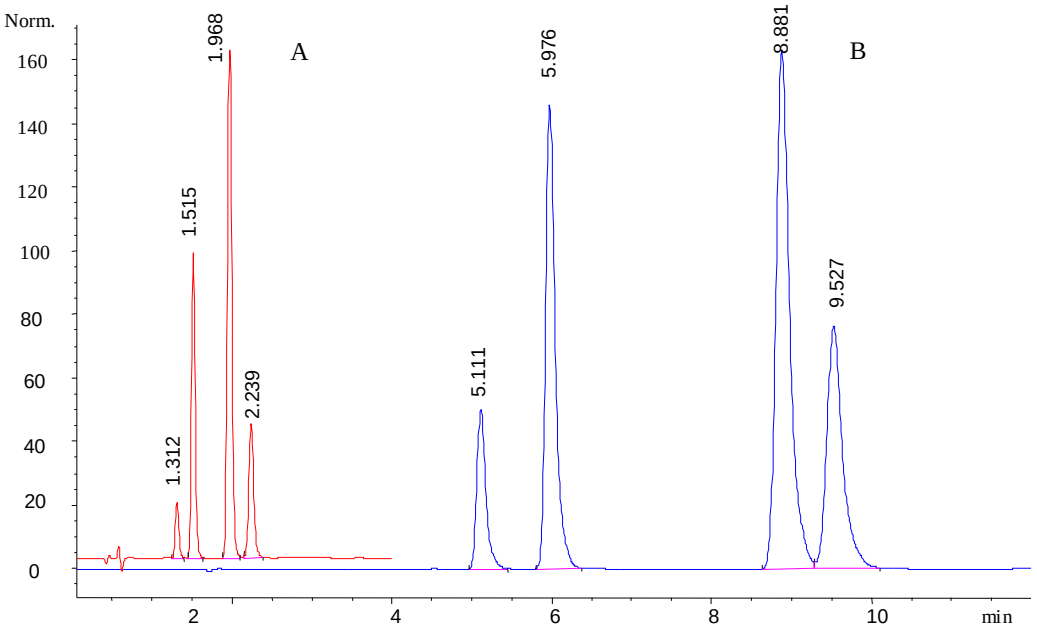

Fig. 2: Comparison of chromatograms of API and three main impurities obtained with original method (A) and new method (B).

For method optimisation, a systematic examination of the mobile phase composition and flow rate was conducted. The flow rate and temperature were increased in increments taking retention times, as well as the resolution between the API and impurity 3 and the pressure into account. An isocratic method using THF- ammonium dihydrogen phosphate (pH3 0.05M) (28:72 v/v), with a flow rate of 1.7mL/min at a temperature of 44°C was found to give a retention time of between 2-3 minutes with satisfactory resolution between all peaks. The detection wavelength was kept constant, as was the pH. As theory suggests, the smaller particles allowed the use of higher flow rates without loss of efficiency, and resolution and peak symmetry were improved. The order of elution was, impurity 1, impurity 2, impurity 3 followed by the API ketorolac tromethamine. A comparison of the run times and chromatography between the original and new methods is shown in Fig. 2.

Validation

The method was validated with respect to parameters, laid out by the international conference on harmonisation (ICH 2002), including linearity, precision, accuracy, specificity, LOD, and LOQ.

Linearity

Each of the impurities and the API gave a linear response over the concentration ranges tested. The mean values of the slope, intercept and correlation co-efficient are given in Fig. 3-6. The impurities were run at a low concentration range 0.22 to 1.54µg/mL while the active ingredient ketorolac tromethamine was run at a high standard range 200 to 800µg/mL. The ICH guidelines state that a correlation co-efficient of 0.99 or over is desirable for linearity studies. All curves had values within this range showing there is a linear relationship across the range for the analytical procedure.

Accuracy

The percent recovery of the linearity samples were calculated and are shown in Table 1. Good recoveries were obtained ranging from 98.83 to 100.22% for the API. The percent relative error was also calculated for each concentration giving RSD values of 0.39 and 1.16% for the API.

Table. 1: Recovery data for the API (ketrolac tromethamine).

Concentration (µg/mL)	% Recovery	% RSD
200	98.9478	0.394456
300	99.3123	0.064155
400	98.8342	0.93
500	100.074	0.431724
600	100.224	0.220464
700	99.8898	1.162308

LOQ and LOD

The LOQ and LOD were determined based on signal-to-noise ratios, where the analytical responses of approximately 10 and 3, respectively, were used. The concentrations found are seen in Table 2. A chromatogram for the LOQ for ketrolac tromethamine can be seen in Fig. 7.

Table. 2: Limits of Detection and Quantification for the API and impurities.

	LOD	LOQ
Impurity 1	0.3 µg/mL	1.0 µg/mL
Impurity 2	0.04 µg/mL	0.09 µg/mL
Impurity 3	0.03 µg/mL	0.06 µg/mL
API	0.03 µg/mL	0.08 µg/mL

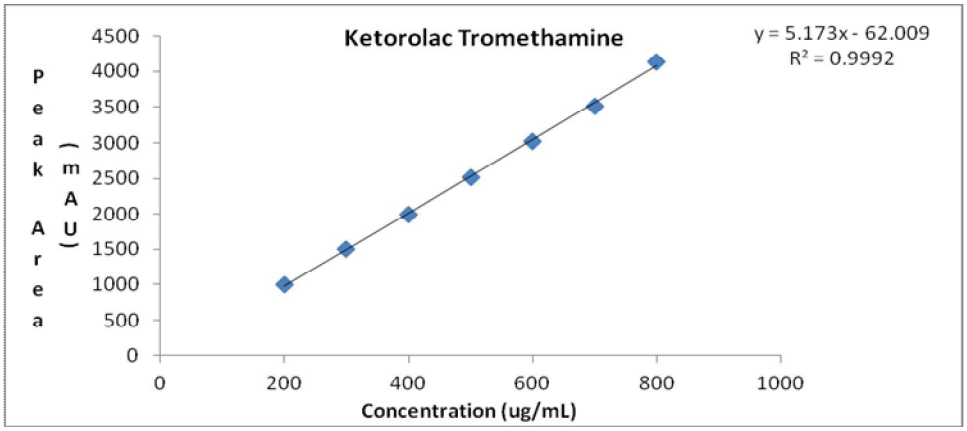

Fig. 3: Calibration curve for ketorolac tromethamine.

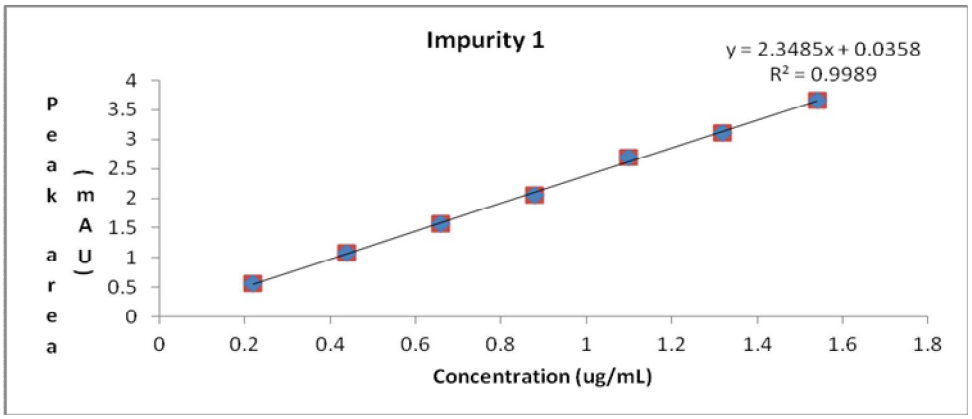

Fig. 4: Calibration curve for impurity 1.

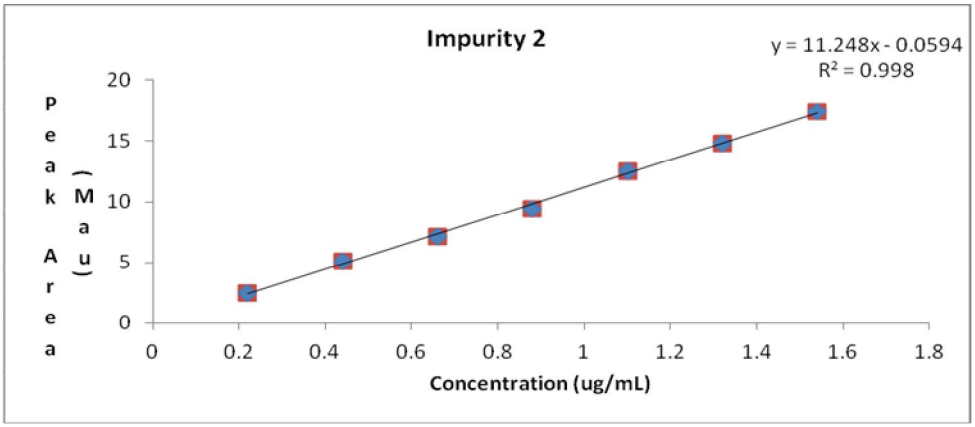

Fig. 5: Calibration curve for impurity 2.

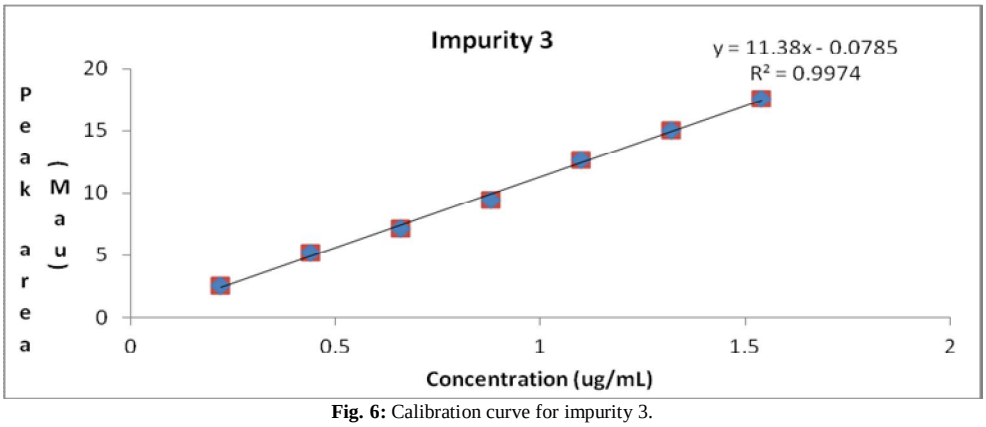

Fig. 6: Calibration curve for impurity 3.

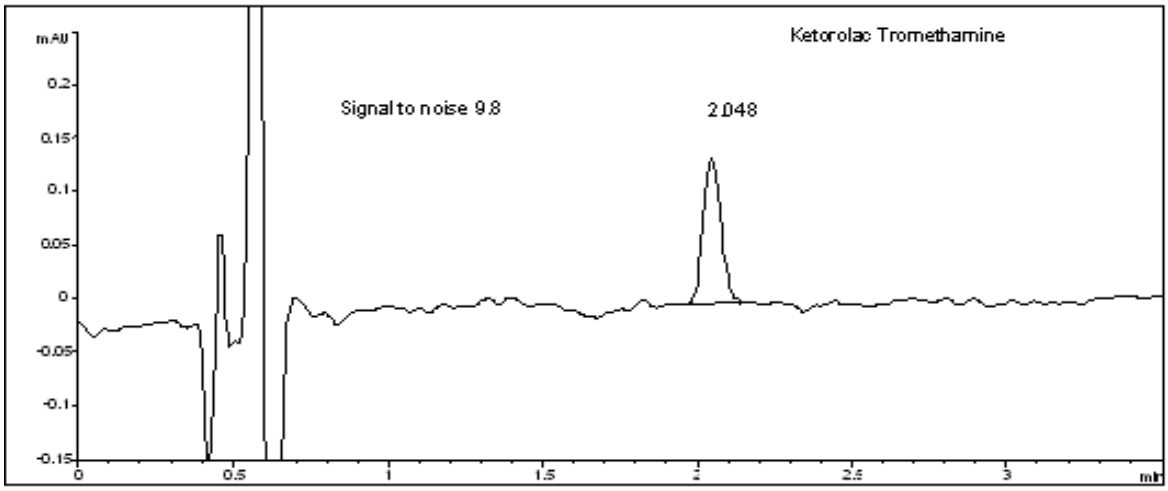

Fig. 7: Limit of quantification of API.

Precision

Repeatability studies were performed by injecting 6 replicates of the API test solution (0.4mg/mL). Repeatability studies on the impurities were performed by injecting 6 replicates of a 4µg/mL standard of the individual impurities. The %RSD values were found to range between 0.43% and 5.56% (Table 3). Results met with the test specifications for the API (1.5%) and the acceptable limit of 10% for the impurities.

Table. 3: Repeatability data for API and Impurities.

Compound	RSD (%)
Impurity 1	5.557509
Impurity 2	2.633431
Impurity 3	5.446363
API	0.433026

Selectivity

Samples of each of the separate impurities were prepared and injected 6 times. A ketorolac tromethamine sample was also injected. All samples had different retention times. The test solution was also injected. All peaks were sufficiently separated and no interference was noted.

Sample Stability

The stability of the samples, stored in clear and amber glassware in ambient temperatures, and in clear glassware in the fridge (4ºC) was tested at five intervals; immediately, after one day, after 7 days, after 28 days and after 42 days. No degradation of the samples occurred under any of the conditions for the first 7 days. Degradation products were seen in the samples stored in the clear glassware at ambient temperatures after 4 weeks. No degradation products were seen in the samples stored in the other conditions. Chromatograms from the clear ambient sample from day 1, day 28 and day 42 are shown in Fig. 8A, B and C.

Method Robustness

Wavelength, pH and mobile phase composition were varied to determine the robustness of the method experimental conditions. At the pH of 2.5 all impurities and the active ingredient were separated but resolution suffered slightly. At the pH of 3, impurity 3 and Ketorolac tromethamine were co-eluting. When the mobile phase was varied, the resolution at 70:30 between the impurity 1 and impurity 2 and the resolution between impurity 3 and ketorolac tromethamine was slightly less than 1.5. At 74:26 the resolution was satisfactory but the retention time was lengthened. There was no noticeable difference between the chromatograms when the wavelength was varied by ±2 nm.

Sub 2 µm Column Method

A review of suitable sub 2 µm packed columns was also undertaken in order to further reduce the time for analysis. An Agilent, zorbax SB-C8, 1.8 µm, 4.6 mm (i.d.) x 50 mm column was found to be suitable giving good resolution between peaks (greater than 1.8), together with reducing the elution time of the individual peaks. The gradient program for this analysis can be seen in Table 4 with the resultant chromatographic plot seen in Fig. 9.

Table. 4: Gradient program for Agilent, zorbax SB-C8, 1.8 µm column (A THF, B Buffer).

Time	% A	% B
0.00	73	27
0.7	62	38
1.00	60	40
2.00	73	27
3.00	73	27

The chromatographic conditions for this analysis utilises a THF-ammonium dihydrogen phosphate (pH3 0.05M) mobile phase with gradient elution at a flow rate of 2ml/min with UV detection at 313nm. The column was heated to 40°C and an injection volume of 3µl was used. The mobile phase was filtered through 0.45µm nylon filters and degassed in an ultrasonic bath prior to use.

The use of a sub 2µm column allows for the shortest elution time for ketrolac tromethamine and its impurities, however at least 5 minutes of a equilibration time is required after the elution of the final peak resulting in an overall greater sample analysis time.

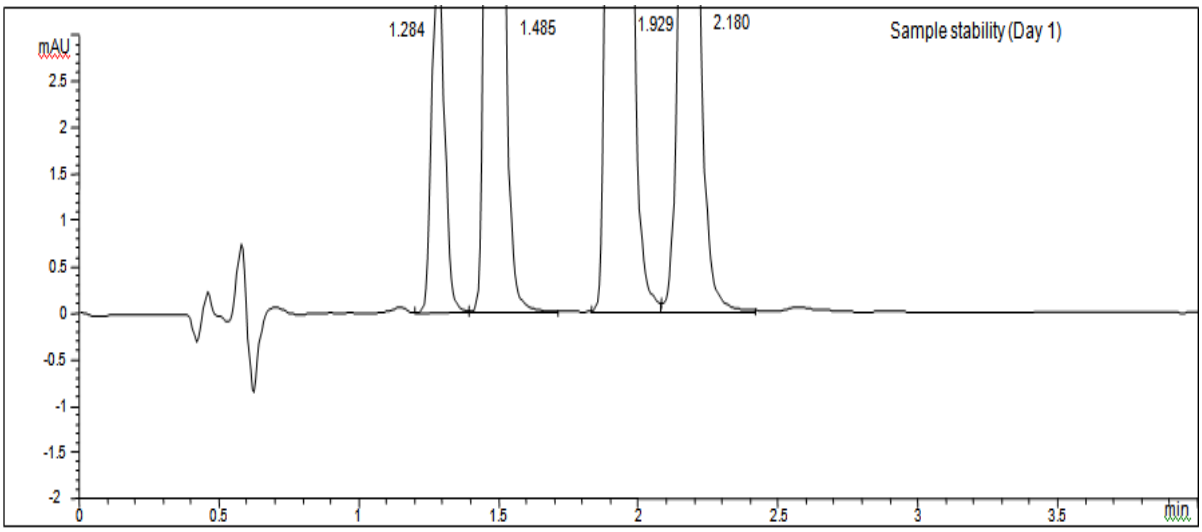

Fig. 8A: Chromatograms of naturally aged API and impurity samples: Day 1.

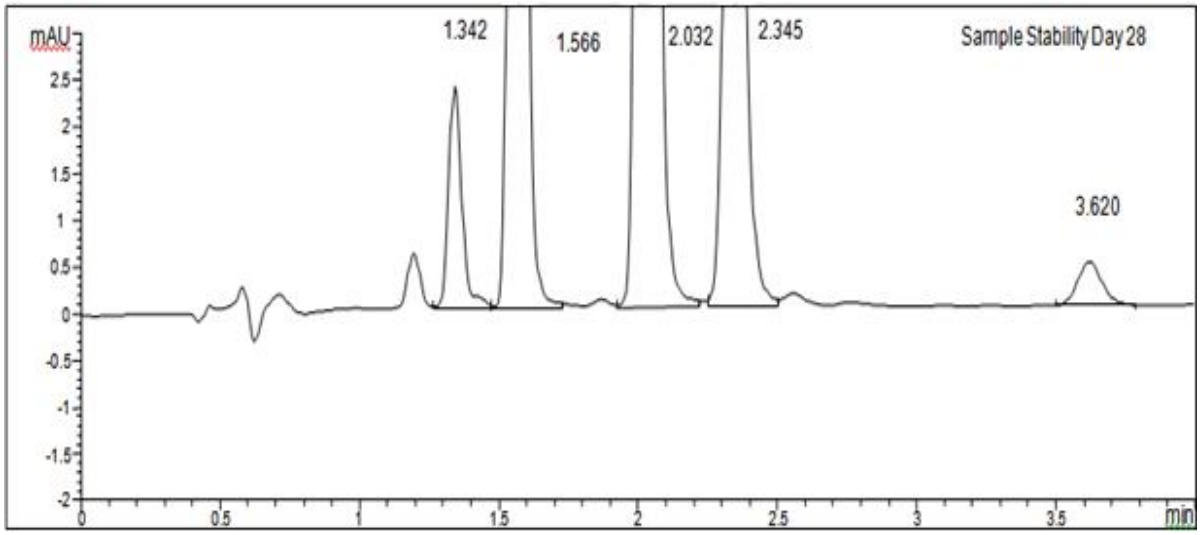

Fig. 8B: Chromatograms of naturally aged API and impurity samples: Day 28.

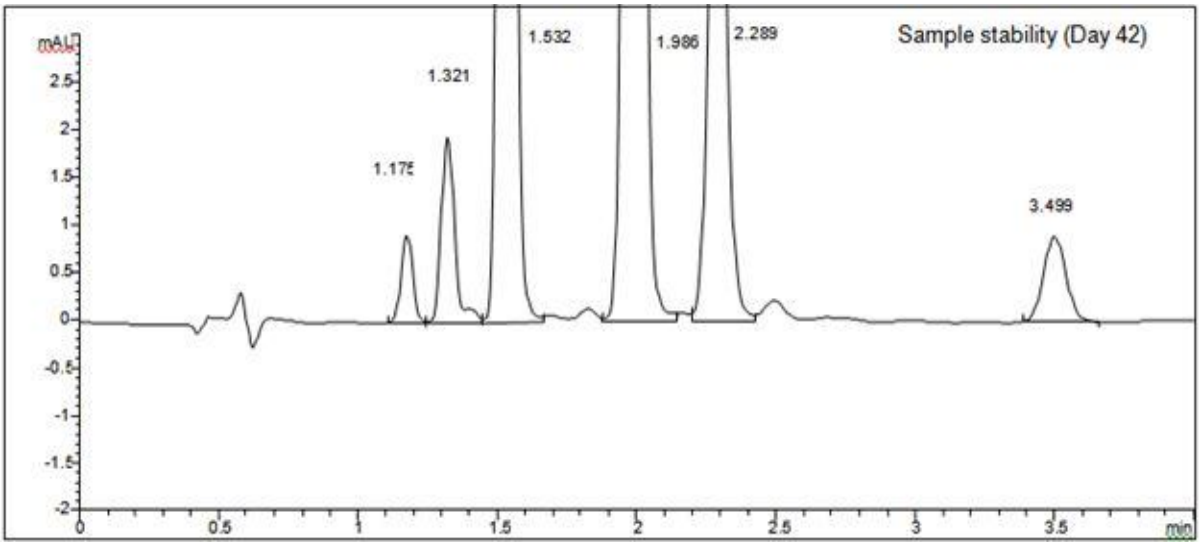

Fig. 8C: Chromatograms of naturally aged API and impurity samples: Day 42.

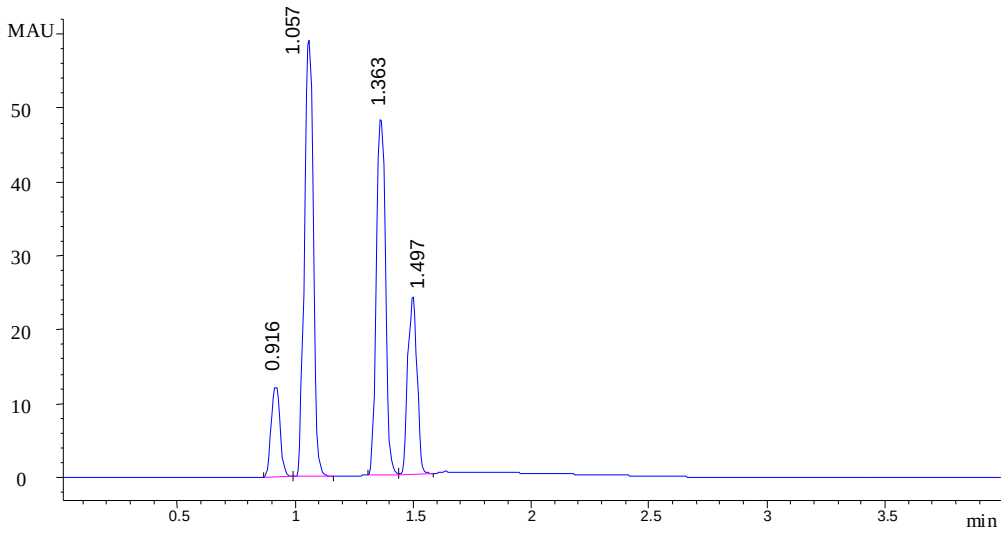

Fig. 9: Chromatogram of the separation of ketorolac and impurities on the Agilent C8, 1.8 µm column.

CONCLUSION

A rapid HPLC method for the determination of ketorolac tromethamine and its main production impurities was developed and validated. It was found that the analysis times could be reduced three-fold in comparison with the current approved European Pharmacopeia method. This is achieved while maintaining resolution and good efficiency. The method was proven to be accurate, precise and capable of satisfactorily separating all impurities in under three minutes. Basic stability studies showed that the samples can be stored without degradation for a period of up to 7 days in clear glassware and up to 42 days in amber glassware. It can be used for the routine analysis of ketorolac tromethamine and its related impurities in bulk API form. Furthermore the lower solvent consumption along with the significantly reduced run time leads to an environmentally friendly economically analytical procedure that allows for the analysis of a large number of samples over a short period of time.

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REFERENCES

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.

Devarajan P, Gore S and Chavan S, HPTLC determination of ketorolac tromethamine, *J. Pharmaceutical and Biomedical Analysis* 2000; 22 (4): 679-683.

De Villiers A, Lestremau F, Szucs R, Gélébart S, David F and Sandra P, Evaluation of ultra performance liquid chromatography Part I. Possibilities and limitations, *J. Chromatography A* 2006; 1127 (1-2): 60-69.

Dongre V, Karmuse P, Rao P and Kumar A, Development and validation of UPLC method for determination of primaquine phosphate and its impurities, *J. Pharmaceutical and Biomedical Analysis* 2008; 46: 236-242.

European Pharmacopoeia 6.0, (2008) Vol.1, pp 2220-2221.

Guillarme D, Nguyen D, Rudaz S and J-L. Veuthey, Recent developments in liquid chromatography—Impact on qualitative and quantitative performance, *J. Chromatography A* 2006; 1149 (1) 20-29.

Ing-Lorenzini K, Desmeules J, Besson M, Veuthey J, Dayer P and Daali Y, Two-dimensional liquid chromatography-ion trap mass spectrometry for the simultaneous determination of ketorolac enantiomers and paracetamol in human plasma: Application to a pharmacokinetic study, *J. Chromatography A* 2009; 1216 (18): 3851-3856.

Jones D and Bjorksten A, *J. Chromatography B: Biomedical Sciences and Applications* 1994; 661(1): 165-167.

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.

Rang H, Dale M, Ritter J and Flower R, *Pharmacology, 6th Ed.*, Churchill Livingstone, Elsevier, (2007) 227-230.

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.

Wang Z, Dsida R and Avram M, Determination of ketorolac in human plasma by reversed – phase high-performance liquid chromatography using solid-phase extraction and ultraviolet detection, *J. Chromatography B* 2001; 755 (1-2): 383-386.

Wren S and Tchelitcheff P, Use of ultra-performance liquid chromatography in pharmaceutical development, *J. Chromatography A* 2006; 119 (1-2): 140-146.