

Simultaneously Determination of the Enantiomers of Ketorolac as well as XBL011003 in Human Plasma with LC/MS

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Overview

A rapid and specific LC/MS/MS method capable of quantifying (*R*)-ketorolac, (*S*)-ketorolac, and XBL011003 in a single experiment, with a lowest limit of quantitative level as low as 5 ng/mL for (*R*)- or (*S*)-ketorolac and 0.1 ng/mL for XBL011003 is described.

In this method, the drug was extracted from plasma using solid phase extraction. The analytes and added internal standards are separated using a chiral column, and detected in both positive and negative ion modes. The method was validated over linear range of 5-500 ng/mL for ketorolac and 0.1-50 ng/mL for XBL011003. Excellent linearity, accuracy, and precision were obtained.

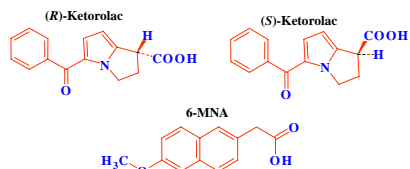
The method has been successfully applied to clinic sample analysis.

Introduction

Ketorolac (Toradol®) is an anti-inflammatory medicine used to treat pain. Studies have shown that the pharmacological activity of ketorolac resides in the (*S*) enantiomer and that the (*R*) enantiomer is pharmacologically inactive. Recently ketorolac was combined with other medicine such as XBL011003 for the treatment of mild to moderately severe post-operative pain.

Many analytical methods have been developed for pharmacokinetic studies or clinical trials¹. However, to support clinical trials in human plasma, a more sensitive and effective method to cover both drug analytes is needed. Since ketorolac is a acidic compound and XBL011003 a basic compound, simultaneously extraction and analysis of these two compounds would be a challenge.

We now report a rapid and specific LC/MS/MS method capable of quantifying (*R*)-ketorolac, (*S*)-ketorolac, and XBL011003 in a single experiment, with extremely low quantitation limit.



Experimental

Sample Preparation

A mixture of 0.5 mL of human plasma sample and 20 μ L of an aliquot internal standard solution was loaded onto a pre-conditioned SPE cartridge. The cartridge was washed with a buffer solution and the drugs and IS were eluted with a polar solvent. The SPE extract was evaporated to dryness under a nitrogen stream, and the residue was reconstituted in reconstitution solvent.

Liquid chromatography:

LC System: Pump Shimadzu LC-10AD
Autosampler Shimadzu
System Controller Shimadzu SCL-10A
Analytical Column: Astec ChiroBiotic T 4.6 x 250 mm Gradient
Flow rate: 1.0-1.5 mL/min
Injection Volume: 5 μ L

Mass Spectrometry

MS System: AB Sciex API-4000
Condition: LC(±)-MS/MS (MRM)
The mass spectrometer was set up for the following transition:
(±)Ketorolac 253.7 \rightarrow 209.6
6-MNA (IS) 214.8 \rightarrow 154.8

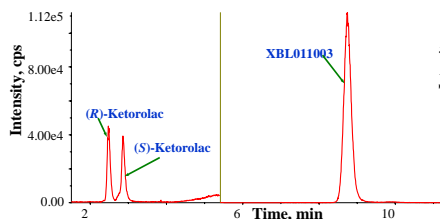


Figure 1. LC(±)ESI-MS/MS (MRM) ion chromatograms of (*R*)-Ketorolac, (*S*)-Ketorolac and XBL011003

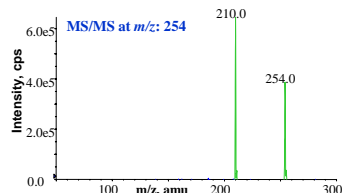


Figure 2. Product ion mass spectrum of Ketorolac.



Figure 3. LC-MS System

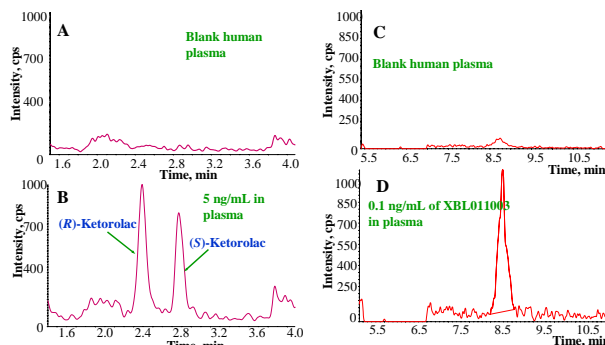


Figure 4. MRM ion chromatograms of (A) blank plasma for Ketorolac ion transition, (B) 5 ng/mL of Ketorolac in plasma, (C) blank plasma for XBL011003 ion transition, and (D) 0.1 ng/mL of XBL011003 in plasma

Results and Discussion

This method can serve following purposes: (1) Quantifying low concentration of (*R*)-ketorolac and (*S*)-ketorolac in human plasma separately; (2) Quantifying (*R*)-ketorolac, (*S*)-ketorolac, and XBL011003 in human plasma in one experiment; (3) Or the method could be modified for quantifying (*R*)-ketorolac, (*S*)-ketorolac and other drugs of interest in human plasma if needed.

Excellent linearity was obtained with correlation coefficient greater than 0.995. The inter-day precision (CV%) and accuracy (RE%) for all QC samples including LLOQ in both (±)ketorolac and XBL011003 were <5% and <12%, respectively (Table I). Three freeze/thaw cycles and ambient temperature storage QC samples for up to 24 hours prior to analysis, appeared to have little effect on the quantitation.

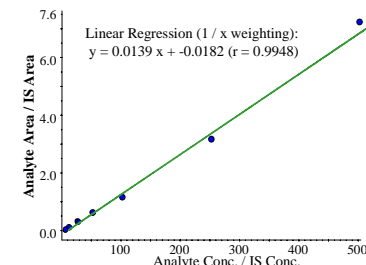


Figure 5. Representative calibration curve for the measurement of (*S*)-Ketorolac in human plasma

(±)Ketorolac						
Calibration Range	5 to 500 ng/mL					
Correlation coefficient (r, 3 batches)	0.9951 to 0.9999					
Accuracy & Precision	QC	Conc. (ng/mL)	Accuracy (RE%)	Precision (CV%)		
				(<i>R</i>)	(<i>S</i>)	(<i>R</i>)
Inter-Batch (n=18)	LLOQ	5	3.60	8.40	11.74	6.14
	Low	15	8.67	2.00	4.64	10.00
	Medium	225	12.00	1.78	2.56	10.35
	High	450	11.11	5.56	3.30	6.32
			Accuracy (RE%)			
Storage Stability			QC-Low	QC-High		
	Condition		(<i>R</i>)	(<i>S</i>)	(<i>R</i>)	(<i>S</i>)
Room Temperature	~ 24 hrs		8.00	-4.00	10.89	-2.44
Freeze and Thaw	-20C, 3 cycles		12.00	-10.00	2.89	-9.78
Extracts in Refrigerator	-4C, 3 days		6.67	-8.67	12.67	9.11
Long-Term	-20C, 44 days		6.67	7.33	-11.11	-1.78

Table I. Human plasma validation results of (±)-Ketorolac

Conclusion

A rapid and specific LC/MS/MS method was developed for quantifying (*R*)-ketorolac, (*S*)-ketorolac, and XBL011003 in a single experiment, with a lowest limit of quantitative level as low as 5 ng/mL for (*R*)- or (*S*)-ketorolac and 0.1 ng/mL for XBL011003.

References

1. Tsina, I., Tam, Y. L., Boyd, T., Rocha, C., and Massey, I. "An Indirect (Derivatization) and A Direct HPLC Method for the Determination of the Enantiomers of Ketorolac in Plasma." *J. Pharm. Biomed. Anal.* 15 (1996) 403-417