Supplementary information

Materials and Methods

Mobile phase preparation

Plasma concentration of IBU from HEC-IBU conjugate **3** was determined using HPLC/UV method. Analysis was carried out using methanol as mobile phase which was filtered and degassed before analysis.

Preparation of standard solution of IBU

Stock solution of IBU was prepared by adding 100 mg of IBU in 100 mL of mobile phase. 10 mL of this standard solution were diluted to 100 mL using mobile phase, filtered through 0.45 μ syringe filter and degassed before analysis. Suitable dilutions were prepared where required.

Chromatographic conditions

Flow rate of mobile phase was varied to optimize chromatographic conditions for determination of IBU. Optimum separation was achieved with flow rate of 1.0 mL min^{-1} at detection wavelength of 264 nm using injection volume of 20 μL for each run.

Method validation

HPLC/UV method was validated using the mobile phase as per reference (Shah et al. 2000).

Precision and accuracy

Precision and accuracy of the developed HPLC/UV method for determination of IBU was determined with help of quality control samples. The quality control samples included different concentrations one of which was within 3 times the lower limit of detection (low quality control sample), one near the middle range (middle quality control sample) and one near the upper range (high quality control sample) of calibration curve of IBU. Ten replicate readings were taken for

each sample and the co-efficient of variance (CV) and the mean were calculated to determine precision and accuracy.

Quality control

Response of aforementioned quality control samples was used to accept or reject the run.

Linearity

Ten different concentrations of IBU were prepared in mobile phase. 20 µL of each concentration were injected and response was measured at 264 nm to plot calibration curve.

Limit of detection (LOD)

Mobile phase was used to prepare suitable dilutions of IBU to known concentrations until signal to noise ratio was two in the final response.

Lower limit of quantification (LOQ)

LOQ was determined using five samples independent of concentration of standards and CV was calculated for each sample at 95% confidence level.

Specificity

Specificity of the developed method was determined using six different samples. No interfering impurity was observed in the chromatograms while the drugs showed optimized retention under the selected conditions.

Stability study

Safety of the mobile phase for IBU was determined by carrying out stability study. The drug did not show any degradation during preparation, handling and recording of the samples up to 72 h.

Results and Discussions

Method validation

HPLC/UV analysis revealed LOQ and LOD values of 2.0 and 0.2 μg mL^{-1}, respectively for IBU from plasma. Between days precision near LOD was used to determine co-efficient of variance (CV) and its value was 0.39%. Accuracy of the developed method for determination of IBU was measured in terms of percent recovery and it was found to be 94.88% (Table S1). The values of performance parameters revealed the validity of developed reverse phase HPLC/UV method for the pharmacokinetic study of HEC-IBU conjugate **3**.

Table S1 Validation parameters for HPLC/UV analysis of IBU from plasma.

No.	Parameter	IBU from HEC-IBU conjugate **3** (Mean)
1	Concentration range	5-40 μg mL^{-1}
2	LOD	0.2 μg mL^{-1}
3	LOQ	2.0 μg mL^{-1}
4	Linear regression co-efficient (r^2)	0.9996
5	Precision Intra-day (RSD)	0.685%
6	Precision Inter-day (RSD)	0.395%
7	Accuracy (% recovery)	94.88%
8	Repeatability (RSD)	0.525%

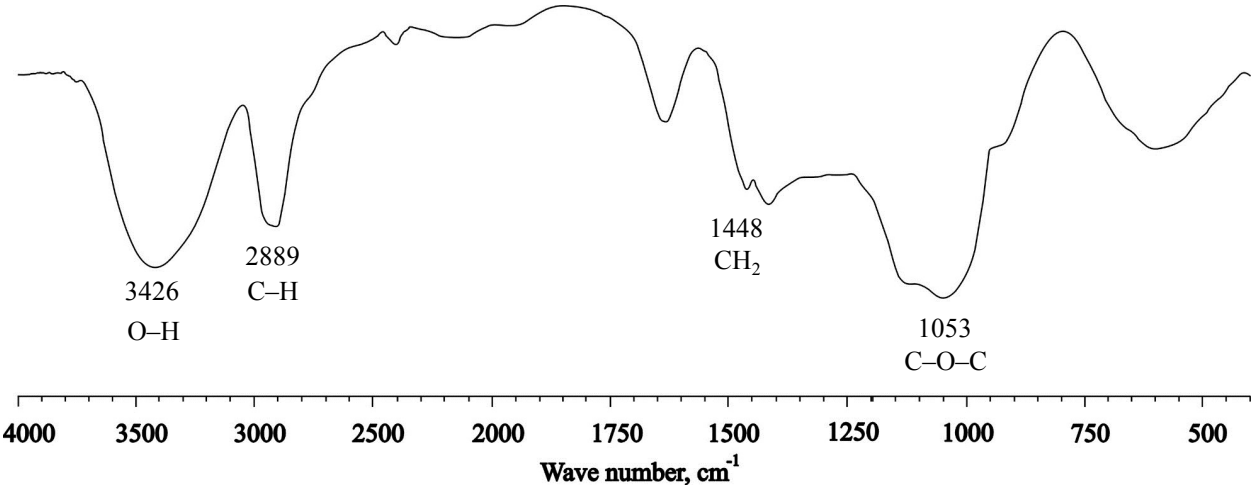

Fig. S1. FT-IR (KBr) spectrum of HEC. Reproduced from Amin et al.[16] with kind permission from Springer Science + Business Media.

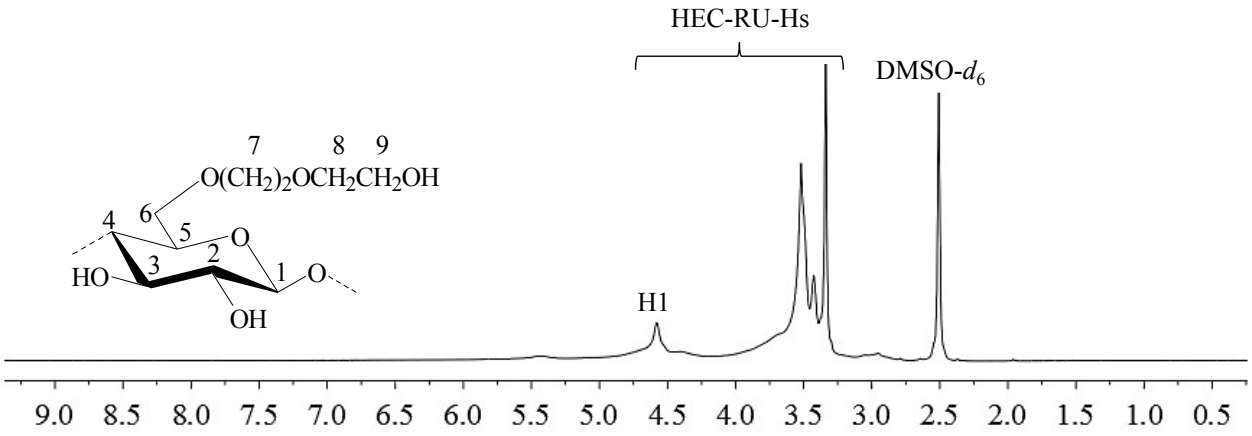

Fig. S2. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) spectrum of HEC. Reproduced from Amin et al.[16] with kind permission from Springer Science + Business Media.