

Rapid Metabolic Stability Screening with Simultaneous Metabolite Profiling Studies of Clozapine in Rat Hepatocytes

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Introduction

The evaluation of the metabolic stability of a new chemical entity is often used as one component of an early ADME screening process. The rapid identification of active/reactive metabolites with a lead compound presents a further challenge to drug discovery. Recent reports have demonstrated an approach for the simultaneous analysis of a parent drug and screening for its metabolites. The ability to separate the metabolites in a very short time along with collecting structural information in a rapid MS duty cycle are critical factors in this approach. In this study, we report on the rapid and simultaneous metabolic stability and metabolite profiling of clozapine in rat hepatocytes using a hybrid triple quadrupole linear ion trap mass spectrometer.

Methods

The metabolite profile of clozapine was evaluated at 1 μ M following incubation with cryopreserved rat hepatocytes at 37°C for 2 hours. LC/MS/MS analysis was performed on an API 4000 Q-Trap Mass Spectrometer with an Agilent 1200 HPLC and HTC PAL autosampler. A gradient separation was completed in 5 minutes using a 50 x 2 mm 2.5 micron Synergi Polar-RP analytical column. An Information Dependent Acquisition (IDA) method was configured using Metabolite ID software to monitor 45 SRM channels for common Phase I and Phase II metabolites. Two additional SRM channels excluded from the IDA method were used to monitor the parent and internal standard. The total scan time for the combined SRM and SRM-IDA-EPI approach was 1.1 seconds.

Preliminary Data

The pooled male Sprague-Dawley rat hepatocytes remained viable for the 2 hour duration of the experiment as determined at 0 and 2 hours by trypan blue exclusion. The average cell viability at 0 hours was 74.0% and at 2 hours was 65.3%. In addition, the results for the metabolic stability of clozapine indicated that the rat hepatocytes were metabolically active with a half life determined from the percent clozapine remaining of $t_{1/2} = 0.364$ hours. When compared to the combined SRM-IDA-EPI method, the corresponding half life for the metabolic stability of clozapine was $t_{1/2} = 0.321$ hours. The results for the metabolic stability in hepatocytes observed by either approach were consistent with previous SRM only results obtained in our laboratory for clozapine stability with different batches of rat hepatocytes ($t_{1/2} = 0.241 \text{ h} \pm 0.103 \text{ h}$, $n=11$). Subsequent EPI data

derived from the combined SRM-IDA approach was then used for the identification and quantitation of potential clozapine metabolites. A total of up to seven potential metabolites were extracted from the EPI spectra of the rat hepatocyte samples when compared to the zero hour control group. In particular, there was strong EPI daughter ion evidence for the oxidation, N-demethylation, methylation and oxidation as well as the oxidative dechlorination of clozapine. The metabolite profiling analysis successfully separated a number of potential glucuronide metabolites as well. Finally, the number and type of metabolites identified using the rapid stability/profiling combined methodology agreed well with our previous internal clozapine studies using more conventional metabolite profiling techniques. The novel screening and metabolite profiling paradigm for clozapine and its metabolites has been applied to our routine drug discovery screening activities.

Novel Aspect

Metabolic stability and profiling of Clozapine in hepatocytes using a simultaneous SRM-IDA-EPI MS method for rapid drug discovery screening.