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INFLOWMATION CHRONICLES
HIGHLIGHTS OF INTERESTING SCIENTIFIC APPLICATIONS

HPLC Post column addition

It is common to add ion suppression solution or derivatizing agent after the HPLC separation and prior to the entrance to the MS. Usually a separate syringe pump with high accuracy and precision with pulseless flow is required. This assures the mixing of the proper amount of post column addition without the destruction of the HPLC's resolution.

LC–MSDetermination ofp-(1-Dimethylamino ethylamino) aniline: a Metabolite of Tribendimidine in Human Plasma

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A sensitive and selective liquid chromatography–mass spectrometric method was developed and validated for the determination of p-(1-dimethylamino ethylamino)aniline (dADT), a metabolite of tribendimidine, in human plasma. The analyte was separated on a Hypersil C18 column (250 · 4.6 mm ID, 5 lm) by isocratic elution with 10 mM ammonium acetate (containing 0.1% triethylamine)-acetonitrile (20:80, v/v) at a flow rate of 1.0 mL min1 and measured by electrospray ionization source in positive selective ion monitoring mode at m/z 178. The weighted (1/x2) calibration curve was linear within a concentration range of 0.5–1,000 ng/mL and displayed a correlation coefficient (r) of 0.9996. The lower limit of quantification was determined to be at 0.5 ng/mL. The inter and intra-day precisions (%RSD) were less than 8% and the extraction recoveries ranged from 84.21 to 85.20%. The developed method was successfully applied to the determination of dADT in human plasma as part of a clinical pharmacokinetic study.

Introduction

Tribendimidine, N,N0-bis[40-(1dimethylamino ethylidene amino)phenyl]-1,4-phenylenedimethylidyne amine, is a new and effective anthelmintic agent which showed high activity against A. lumbricoides and Necator americanus without mutagenic and clastogenic effects compared with other anthelmintic agents [1].

KDS Model 100 syringe pump

Key features for this application:

- **High precision and accuracy**
- **Smooth flow**

1221 million people are estimated to be infected with Ascaris lumbricoides which is the most common intestinal nematode. This is followed by an estimated figure of 795 million people who are infected withTrichuris triciura, and furthermore, 740 million people are expected to show infections by hookworms [2]. Tribendimidine shows promising potential because it is considered safe with a broad range of activities. Some studies reported on the clinical application and pharmacodynamics of this parent compound [3,4] but few were based on the investigation of human metabolism and pharmacokinetic profiles. Knowledge of pharmacokinetic processes can help to explain and predict a variety of events related to efficacy and toxicity. It is therefore important to investigate the pharmacokinetics of tribendimidine for further evaluation of its clinical use. Some pharmacokinetic studies have been carried out in our laboratory and p-(1-dimethylamino ethylimino)aniline (dADT) has been identified as a metabolite of tribendimidine in human plasma after administration of enteric-coated tablets. This study describes a simple, validated, rapid and sensitive LC–MS method for the determination of dADT in human plasma within a tribendimidine pharmacokinetic study in healthy Chinese volunteers. Experimental Chemical and Reagents dADT standard was provided by Xinhua.

Application to Pharmacokinetic

Study in Humans

Thirty healthy Chinese volunteers (50% males and 50% females), aged 23–27 years, were selected for the study after clinical assessment of their health status. The protocol has been approved by the Ethic Committee of Qilu Hospital, and the study was conducted in accordance with the Declaration of Helsinki and was based on written informed consents. The volunteers were randomly divided into 3 groups of 10 and 200, 400 and 600 mg tribendimidine entericcoated tablets were orally administered with 200 mL water in the morning after a 10 h fasting period. Blood samples (4 mL) were collected in heparinized tubes before (0 h) and 0.5, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12 and 24 h after administrations. Plasma was separated, collected and stored at 20 C for analysis. Drug and Statistic (DAS, version 2.0, by Sun Ruiyuan et al. [5], China) was used to fit the compartmental model of dADT in human and calculate the main pharmacokinetic parameters as half life ($T_{1/2}$)and area under the plasma concentration versus time curve (AUC024 and AUC0xx). The peak plasma concentration (Cmax) and its corresponding time (Tmax) were observed values.

Results and Discussion

Optimization of LC–MS Condition and Sample Preparation According to the chemical constitution of dADT, electrospray ion source and positive selective ion monitoring mode were selected to obtain strong signal responses and high sensitivity. Under the selected source conditions used, the protonated molecule peak at m/z 178 appeared in the full scan spectra of dADT. A fragment voltage of 90 V was selected in order to generate the best signal response. Little is known about the physicochemical

properties of dADT. A liquid– liquid extraction method was evaluated using chloroform, acetic ether and methylbenzene but with unsatisfactory recoveries when compared with protein precipitation.

Acetonitrile was selected as the protein precipitant and sodium chloride was used to separate acetonitrile from the aqueous layer and to improve extraction. When the chromatographic conditions were optimized it was found that the use of acetonitrile resulted in improved retention times and lower background noise when compared with methanol. The use of 10 mM ammonium acetate improved ionization of the analytes and triethylamine was used to improve peak shapes. Under the optimized LC–MS conditions, no interferences of endogenous compounds were observed.Ion suppression effects were investigated during analysis [6]. Blank plasma samples were extracted and injected onto the Hypersil column, while 300 ng MI1 standard solution of dADT was infused post-column through a zero dead volume tee using a KD Scientific syringe pump (Holliston, MA) at a flow rate of 2 mL/h. No ion suppression from blank plasma was observed.

Conclusion

A sensitive and selective LC–MS method was developed and validated for the determination of dADT, the metabolite of tribendimidine, in human plasma. This has been successfully applied to Pharmacokinetic studies in healthy Chinese volunteers after oral administration of tribendimidine enteric-coated tablets.

Reference

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