ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Apr；21 (4)：306 – 308
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Determination of idebenone in plasma by HPLC/MS

HU Pei[1]，JIANG Ji，XIE Hong-Hong （*Clinical Pharmacological Research Center，Peking Union Medical College Hospital，Peking Union Medical College，Beijing 100730，China*）

KEY WORDS idebenone；pharmacokinetics；high pressure liquid chromatography；mass spectrum analysis；clinical pharmacology；benzoquinones

ABSTRACT

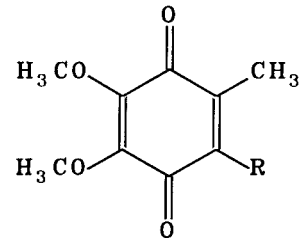
AIM：To develop a sensitive method for the analysis of idebenone [Ide，6-(10-hydroxydecyl)-2，3-dimethoxy-5-methyl-*p*-benzoquinone] in human plasma. **METHODS**：The concentrations in plasma were determined by a high performance liquid chromatography-mass spectrometry coupled with an atmosphere pressure chemical impact ion source method (LC/MS-APCI). **RESULTS**：The chromatograms indicated a good separation of the analytes and there was no interference by other compounds. The coefficients of determination of the calibration curves were above 0.999. The calibration range was 20 – 600 $\mu g \cdot L^{-1}$. Peak plasma idebenone concentration (C_{max}) of (316 ± 85) $\mu g \cdot L^{-1}$ was achieved within (96 ± 34) min (T_{peak}) after an oral dose of 30 mg. Lower limit of quantitation of the method for Ide determination was 20 $\mu g \cdot L^{-1}$. **CONCLUSION**：The advantages of using LC/MS-APCI technique include better sensitivity，higher selectivity，and less time consumption，compared with HPLC method.

INTRODUCTION

Idebenone [Ide，6-(10-hydroxydecyl)-2，3-dimethoxy-5-methyl-*p*-benzoquinone] is a benzoquinone compound structurally unrelated to other agents undergoing investigation for the treatment of senile cognitive disorders. This drug has been studied in patients with mild to moderate senile dementia of the Alzheimer type and vascular dementia[1]. Improvements due to drug treatment were reported in all trials in Japanese studies[2].

The pharmacokinetic properties of idebenone have been investigated in a very limited number of studies conducted mostly in healthy volunteers[1]. Most investigators used HPLC methods which involved many steps in sample preparation and a 70-min long HPLC chromatographic process to assay idebenone and its metabolites in human serum，plasma，and urine. However，and especially for pharmacokinetic studies，there is a need to improve HPLC methods in terms of ease of sample handling and/or analysis time，as a large number of samples have to be analyzed.

The purpose of the present study was to develop a rapid and sensitive method for the quantitative determination of Ide in plasma for use in a comparative bioavailability study after a single oral dose (30 mg) of Ide.



R=$(CH_2)_9CH_2OH$ Idebenone
R=$(CH_2)_9OH$ QSA-9

MATERIALS AND METHODS

Chemicals Ide and QSA-9 used as the internal standard substance were supplied by Takeda Chemical Industries，Ltd. Acetonitrile (HPLC grade) was purchased from Jinghang Chemical Industries，Beijing，China. Chloroform and hexane are the products of Beijing Chemical Reagent Factory，Beijing，China. Other reagents (AR) were purchased from Beijing Chemical Reagent Factory，Beijing，China.

Instrumentation LC-MS was carried out on a modular LC/MS (APCI) system consisting of a Waters 510 pump with a Supelco C_{18} column (5 μm，250 mm × 4.6 mm ID)，a Trio 1000 quadrupole mass spectrometer (Finnigan，UK) equipped with an atmospheric pressure

[1] Correspondence to Prof HU Pei. Phn 86-10-6529-6573. Fax 86-10-6512-4875. E-mail peihu@public3.bta.net.cn
Received 1999-09-27 Accepted 1999-09-30

chemical ionization interface. Analysis was performed with a fixed filament emission current and electron energy of 70 eV. The ion source temperature was 200 ℃ and the LC interface was held at 250 ℃. A high purity nitrogen was used as the nebulizing gas under 600 ℃ of prob temperature.

Quantification of Ide in plasma was carried by SIR (Selected Ion Recording) LC-MS. The protonated molecule ion $(MH)^+$ of the analyte and internal standard (at m/z 339 and 325, respectively) were monitored, and the ratio of responses thus obtained was related to a standard curve prepared by the analysis of plasma containing varying amounts of Ide over the range of 20 – 600 $\mu g \cdot L^{-1}$ and a fixed quantity (250 $\mu g \cdot L^{-1}$) of the internal standard named QSA-9, a derivative of Ide.

Assay procedure An aliquot of plasma (1 mL) was mixed with 25 μL internal standard (10.0 $mg \cdot L^{-1}$), 1 mL distilled water and hydrochloric acid 2 mL 6 $mol \cdot L^{-1}$. After a 15-s vortex, the mixture was heated up to 70 ℃ in a water bath for 20 min. The incubation mixture was extracted with 7 mL of a mixture of chloroform and hexane (4∶6, v/v). The organic layer was separated and evaporated to dryness under nitrogen gas stream at 50 ℃. The residue was reconstituted by 100 μL acetonitrile. A 95 μL portion of this solution was injected onto the HPLC column. The column was eluted with a mobile phase of 80 % acetonitrile made up with deionized water at a flow rate of 1 $mL \cdot min^{-1}$.

Intra- and inter-assay precision The reproducibility of the method was determined with human plasma samples containing Ide. Ide 20, 100, 300, and 600 $\mu g \cdot L^{-1}$ were added to the plasma with certain amount (250 ng) of internal standard, and the samples were measured according to the assay procedures in this assay method. A set of calibration standards in plasma were analyzed with 4 concentrations of Ide 20, 100, 300, and 600 $\mu g \cdot L^{-1}$ in plasma. The accuracy was assessed by calculating the percentage deviation between the found and given concentrations. The intra-assay variability of the method was determined using the coefficient of variation of replicate assays ($n = 5$) for each of the 4 concentrations on a single occasion.

On 5 separate occasions the samples at four concentrations (20, 100, 300, and 600 $\mu g \cdot L^{-1}$) were assayed with a set of calibration standards. The inter-assay precision was determined as the coefficient of variation for each set of the 4 concentrations ($n = 5$).

Recovery The relative recovery of Ide in plasma at four concentrations was determined by comparing the response obtained from extracted plasma sample to which were added known amounts of Ide (20, 100, 300, and 600 $\mu g \cdot L^{-1}$, respectively) with the response obtained from the calibration standards prepared by the same method.

Specificity Samples from human plasma taken from 10 subjects were tested to determine whether endogenous components would interfere with the analysis.

RESULTS

Sample mass chromatogram of Ide 100 $\mu g \cdot L^{-1}$ in plasma with internal standard (250 $\mu g \cdot L^{-1}$) is presented in Fig 1. The peak area ratio of Ide and internal standard was linear over the calibration range (20, 50, 100, 300, 600 $\mu g \cdot L^{-1}$) without any endogenous interference from the plasma. Weighted linear regression was used in constructing the calibration lines. Plasma concentrations of Ide were calculated directly from a calibration curve. Such standard graphs from a set of measurements proved the linearity of the method. For concentrations up to 600 $\mu g \cdot L^{-1}$ in all cases there is good linearity, $Y = 0.003637X - 0.020145$, with coefficients of correlation (r) always between 0.996 – 1.000 ($n = 11$).

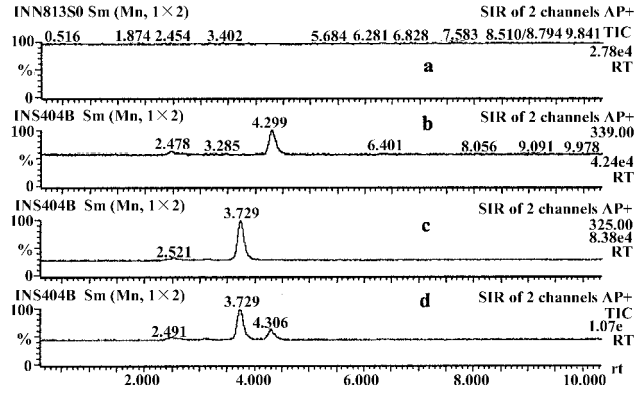


Fig 1. MS chromatogram of Ide in plasma analyzed by LC/MS. a: blank plasma sample without Ide and IS, total current ion. b: blank plasma sample with Ide 100 ng and IS 250 ng added, protonated ion of Ide: m/z 339. c: blank plasma sample with Ide 100 ng and IS 250 ng added, protonated ion of IS. m/z 325. d: blank plasma sample with Ide 100 ng and IS 250 ng added, total current ion.

A high precision and a high accuracy (intra-day and inter-day) variations were obtained with all concentrations 20, 100, 300, and 600 $\mu g \cdot L^{-1}$ (Tab 1).

The lower limit of quantitation for Ide set as a

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Apr；21 (4)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Tab 1. Variation of Ide detection ($n = 5$).

Drug/ $\mu g \cdot L^{-1}$	Intra-assay $\bar{x} \pm s$	CV %	Inter-assay $\bar{x} \pm s$	CV %
20	19.93 ± 0.16	0.8	20.5 ± 0.9	4.3
100	95.9 ± 2.8	3.0	94.4 ± 2.8	3.0
300	304 ± 7	2.3	325 ± 16	4.8
600	615 ± 30	5.0	614 ± 25	4.0

parameter for sensitivity was 20 $\mu g \cdot L^{-1}$ on the basis of the peak approximately 7 times than that of background. The mean relative recovery of Ide from plasma at concentrations of 20, 100, 300, and 600 $\mu g \cdot L^{-1}$ was 99.7 % ± 0.8 %, 95.9 % ± 2.8 %, 101.2 % ± 2.3 %, and 102 % ± 5 %, respectively ($n = 5$).

DISCUSSION

A sensitive, specific, and rapid LC/MS assay was developed for the evaluation of Ide from 1 mL of plasma using Selected Ion Recording that monitored the protonated molecule ion $(MH)^+$ of the analyte and internal standard at m/z 339 and 325, respectively. The LC/MS method described was selective and no endogenous interfering peaks were visible in blank plasma. The most important advantage of this method is less time consumption. The analysis time for one sample has been reduced dramatically from 70 min reported by Japanese (data not published) to 5 min in this study. The sample pretreatment procedures in this study have been slightly improved in terms of saving time for sample preparation.

The calibration plot of peak area ratio (Ide/QSA-9) is linear over the range 20 to 600 $\mu g \cdot L^{-1}$ and the method, calculated from the calibration curve, shows good precision.

The limit of quantitation of the assay was 20 $\mu g \cdot L^{-1}$, which is obviously lower than that reported by Parnetti[3] and is about 1/16 of peak plasma Ide concentration (C_{max}) detected in healthy volunteers after an oral dose of 30 mg Ide.

The data presented on calibration curves and quality control samples indicate that the reported Ide plasma concentrations are reliable. The method reported here shows good characteristics (selectivity, linearity, sensitivity, precision, and less time consumption) and is adequate for the evaluation of Ide in plasma, in pharmacokinetics studies over the collection period, in order to clearly define the absorption and elimination phase of Ide (Fig 2, data not published).

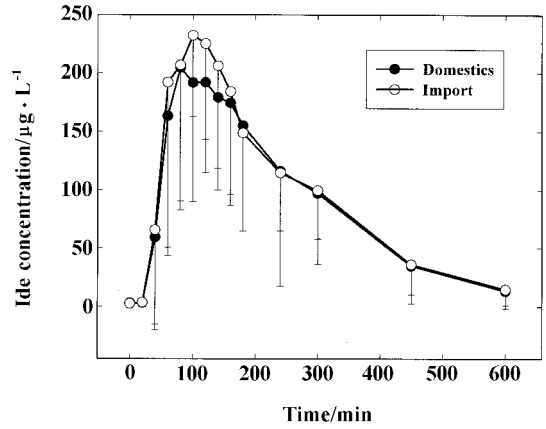


Fig 2. Concentration-time curve after oral administration of Ide. $n = 10$ volunteers. $\bar{x} \pm s$.

ACKNOWLEDGMENT This work was supported by Tianjin Takeda Pharmaceutics, Tianjin, China.

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HPLC/MS 法测定血浆艾地苯醌

胡 蓓[1]，江 骥，谢宏红 （中国协和医科大学北京协和医院临床药理中心，北京 100730，中国）

关键词 艾地苯醌；药物动力学；高压液相色谱法；质量光谱分析；临床药理学；苯醌类

目的：建立测定艾地苯醌(Ide)血药浓度的灵敏方法. **方法**：HPLC/MS 联用仪测定 Ide 血药浓度. 离子源采用大气压化学电离源(APCI)，MS 扫描方式为选择离子检测(SIR). **结果**：血浆中物质在本试验的 LC/MS 条件下对 Ide 和内标 QSA-9 的分离测定无任何影响. 血浆中 Ide 浓度在 20－600 $\mu g \cdot L^{-1}$ 范围内，浓度与峰面积有良好的线性关系，方法的最低检测浓度以 s/n = 7∶1 计为 20 $\mu g \cdot L^{-1}$. **结论**：本法具有非常强的特异性，对 HPLC 柱分离效率的要求不高，分析所需时间短.

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