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Original Article

Determination of Isosorbide Dinitrate in Serum by Gas Chromatography with New Generation of Electron Capture Detector and its Application in Pharmacokinetic Study

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Abstract

Isosorbide dinitrate (ISDN) is an effective drug in treatment of angina pectoris. In this study a new generation of electron capture detector (non-radioactive) with a short, non-polar and wide-bore column was used for analysis of ISDN in human serum. ISDN was extracted from serum by a mixture of ether and ethyl acetate and concentrated at room temperature. The method was linear between 5-50 ng/mL. Recovery and accuracy were 99-108% and greater than 90%, respectively, and inter-day precision was lower than 13%. Pharmacokinetic parameters were analyzed after oral administrations of Isocor 40 mg sustained release tablet in comparison with Isoket Retard 40. The statistical results obtained from comparison of C_{max}, T_{max} and AUC parameters showed no significant difference between these two products and therefore they were reported to be bioequivalent. Further more, the method used was found to be sensitive and accurate in pharmacokinetic studies.

Keywords: Isosorbide dinitrate; Gas chromatography; Electron capture detector; Pharmacokinetic; Serum.

Introduction

Isosorbide dintrate (ISDN) is most commonly used in prophylactic treatment of angina pectoris (1). During the last years, different analytical methods have been performed for analysis of ISDN in human serum (2-5). Due to the low levels of ISDN after oral consumption; the method used for analysis should be highly sensitive and selective. Gas chromatography methods based on the use of electron capture detector, have been found to produce the greatest reproducibility and consistency for analysis of ISDN and its mononitrate metabolites (6-9). They have been widely used in pharmacokinetic studies of different formulations of ISDN (10, 11).

A new generation of electron capture detector that is safer, simpler and more stable in terms of sensitivity (no need for reconditioning of detector) than older versions, was applied in this study. A short, non-polar and wide-bore column, with a modified sample preparation method, was used. This method seem to be reliable in pharmacokinetic studies.

Experimental

Materials

Product "C": Isocor® 40 mg tablet [Arya Pharm Co. (Iran)].

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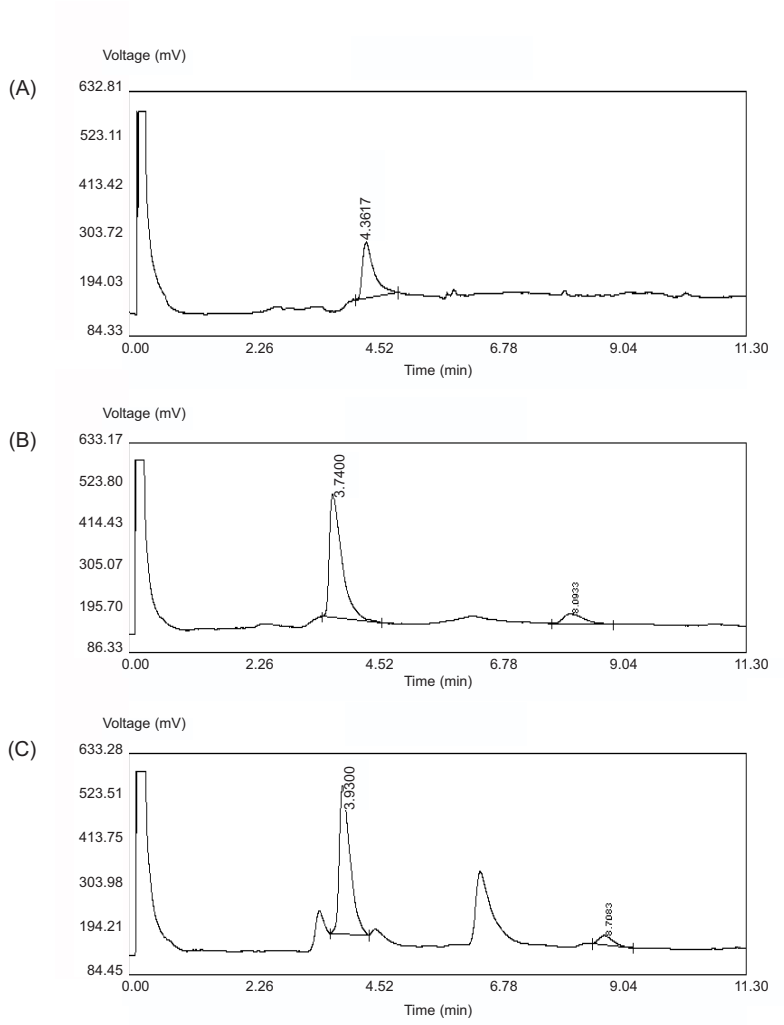

Figure 1. (A) Typical chromatogram of blank serum spiked with 0.7 µg nitroglycerin as the internal standard. **(B)** Chromatogram of blank serum spiked with 0.7 µg nitroglycerin and 50 ng isosorbide dinitrate. **(C)** Chromatogram of the serum sample of a male volunteer, 6 h after administration of 40 mg ISDN.

Product "K": Isoket® retard 40 mg tablet [Schwarz pharma (Germany)].

Isosorbide dinitrate analytical standard and nitroglycerin, as internal standard, were provided by Arya Co. (Iran).

Ethyl acetate extra pure and *t*-butyl methyl ether extra pure were from Merck, Darmstadt (Germany).

Equipments

Younglin model M600D GC, equipped with a VICI electron capture (pulsed discharge) detector was used in this study.

Fused silica, wide bore column coated with polydimethylsiloxane (TRB-1, 10 m × 0.53 mm, film thickness 2.65 µm, Teknokroma, Spain).

Authchro-2000 software was used to acquire and process the data obtained.

Sample preparation

To 1 mL of serum, 0.2 mL of a solution of 3.5 µg/mL nitroglycerin (IS) in ethyl acetate was added. The sample was gently mixed, then extracted with 5 mL of a solvent mixture containing ethyl acetate and *t*-butyl methyl ether (a ratio of 1:4) for 5 min. After 10 min centrifugation, the upper organic layer was transferred into a 15 mL centrifugal tube and evaporated to dryness under a gentle stream of N_2 at room temperature. The dried residue

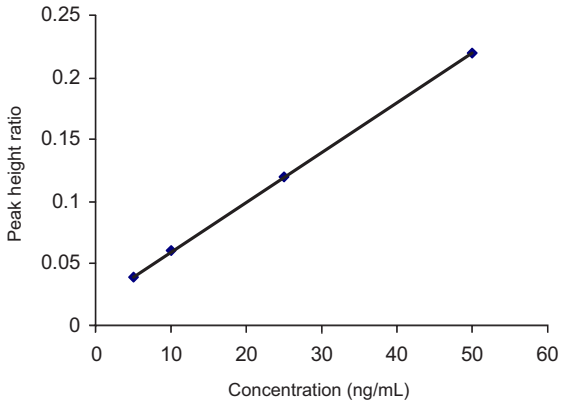

Figure 2. Calibration curve for determination of ISDN in serum by GC.

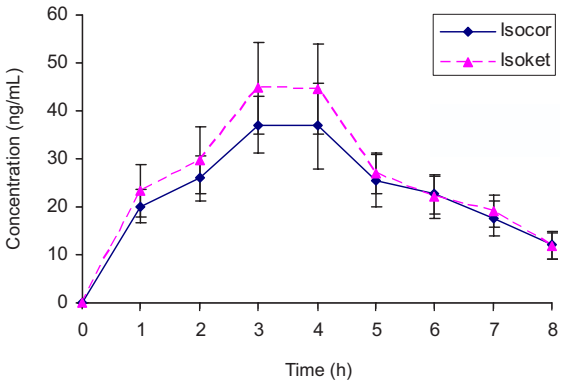

Figure 3. Mean isosorbide dinitrate plasma concentration-time profile in 12 human volunteers after oral administration of a 40 mg ISDN tablet. Each data point represents mean ± SEM.

reconstituted in 100 µL of ethyl acetate and 3µL of this sample was injected, using the deactivated injector liner.

Preparation of standard samples and construction of the calibration curve

Using a 100 ng/mL isosorbide dinitrate in ethyl acetate stock solution, the following standard solutions were prepared in blank serum: 1, 2.5, 5, 10, 25 and 50 ng/mL. They were extracted and injected into GC, based on the method described in sample preparation.

GC system

Helium was used as the carrier gas, with a flow rate of 30 mL/min. Three percent xenon in helium (dopant gas), with a flow rate of 4 mL/min, was employed as the electron source for detection of ISDN and internal standard. The inlet temperature was set at 150°C and the electron capture detector at 200°C. The initial column temperature was 110°C. After 1 min., the temperature was gradually raised to 140°C at a rate of 15°C/min, and this temperature was maintained for 5 min.

Blood sampling

Twelve healthy normotensive (20-52 years) volunteers participated in this study. The protocol was approved by the institutional ethical committee and the volunteers provided written informed consent. They were judged to be in good health on the basis of clinical history, physical and routine laboratory examination. No medication was taken by the subjects from 1 week prior to the study. One tablet of either product "C" or product "K" was administrated in a random cross-over manner to each subject in two different days, with wash out periods of two weeks. Blood samples (10 mL) were drawn in non-heparinized tubes at 0, 1, 2, 3, 4, 5, 6, 7 and 8 h post drug administration. Samples were allowed to clot for 15 min and serum separated and stored frozen at -20°C until the time of assay.

Data treatment

By application of the peak height ratio of the ISDN chromatogram versus internal standard

Table 1. Recovery and accuracy of isosorbide dinitrate in spiked serum.

Amount added (ng/mL)	n	Amount after addition (mean ± SD)	Recovery (%)	Accuracy (%)	C. V. (%)
5	2	5.30 ± 0.65	106	6.03	12.24
10	5	10.82 ± 0.78	108	8.17	7.18
25	7	25.55 ± 2.39	102	2.22	9.37
50	8	49.5 ± 1.27	99	-1.01	2.56

Table 2. Statistical Comparison of pharmacokinetic parameters.

Parameter	Mean ± SE (C)	Mean ± SE (K)	Analysis of varaiance
K_e	0.311 ± 0.05	0.43 ± 0.07	N. S. (P = 0.17)
$T_{1/2}$ (h)	2.99 ± 0.52	2.24 ± 0.52	N. S. (P = 0.32)
T_{max} (h)	3 ± 0.3	3.09 ± 0.28	N. S. (P = 0.83)
C_{max} (ng /mL)	44.38 ± 7.78	55.18 ± 9.56	N. S. (P = 0.39)
$AUC_{(0-\infty)}$ (ng /mL.h)	191.9 ± 35.91	213.98 ± 37	N. S. (P = 0.67)
$AUC_{(0-\infty)}$ (ng /mL.h)	253 ± 51.58	259 ± 43.89	N. S. (P = 0.92)

and the standard curve, concentration of the samples were calculated.

Accuracy was expressed as the mean% {[(mean measured concentration) / (expected concentration)] ×100}. Precision was calculated as the inter-day coefficient of variation [C. V.% = (SD/mean) ×100].

Standard kinetic parameters (C_{max}, T_{max} and AUC) were calculated using the DKNT software (12).

Comparative statistical studies on the bioequivalence between the two formulations were performed, using the variance of analysis. A P-value of less than 0.05 was considered as significant.

Results and Discussion

The GC method used was found to be sensitive, accurate and reproducible.

GC chromatograms of blank serum, human serum spiked with ISDN and sample obtained from a volunteer 3 h after drug administration have been shown in Figure 1.

Retention times of nitroglycerine and Isosorbide dinitrate were found to be around 4 and 9 min, respectively. Standard curve was linear between 5-50 ng/mL ($y = 0.004x \pm 0.0194$, $r^2 = 0.9997$) (Figure 2). The minimum limit of detection was 1 ng/ml.

We found that it is necessary to replace the inactivated glass inlet in the injector by a new one after about 60 injections; otherwise the peak of ISDN would be disappeared.

The results of recovery and precision between 5-50 ng/mL were 99-108% and 2.56-12.24%, respectively, and are shown in Table 1.

The mean serum concentrations of 12 volunteers after administration of either of the two products were calculated and the results have been shown in Figure 3.

Different pharmacokinetic parameters including C_{max}, T_{max} and AUC, for each volunteer after administration of either of the two products were calculated and the mean values for the results obtained are given in Table 2. As could be seen, on average 3 ± 0.30 h after administration of 40 mg Isocor® and 3.09 ± 0.28 h after administration of 40 mg Isoket®, the maximum blood levels had been reached. C_{max} for the two products, as well as AUC, were comparable.

There was no significant difference between these two products. In conclusion, the method adopted in this study, including includes of sample preparation and the use of GC with a new generation electron capture detector, was found to be applicable for determination of nitrated drugs (e. g. isosorbide dinitrate or nitroglycerin) in serum and plasma samples.

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