Determination of Isosorbide Dinitrate and its Two Mononitrate Metabolites in Human Plasma and Saliva Through Gas-Liquid Chromatography with Electron-Capture Detection*

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**Summary.** Two sustained-release formulations of isosorbide dinitrate (ISDN) were administered to eight healthy male subjects and plasma and saliva samples were analysed. The determination of ISDN and its two metabolites 2-isosorbide mononitrate (2ISMN) and 5-isosorbide mononitrate (5ISMN) was carried out after extraction through gas chromatography with an electron-capture detector. Different packed and capillary columns were tested and optimal chromatographic and extraction parameters for the measurement were worked out. A dose of 120 mg ISDN sustained-release preparation results in a plasma concentration of 1 - 42 ng/ml ISDN, 1 - 76 ng/ml 2ISMN and 12 - 475 ng/ml 5ISMN, respectively, during 48 h after drug administration. Good saliva-plasma correlation was obtained only for 5ISMN. Plasma samples should not come into contact with plastic materials in order to avoid losses of ISDN and mononitrate due to adsorption. The relative standard deviation for ISDN and 2ISMN was found to be ± 3 - 7% and for 5ISMN ± 2 - 5%.

**Introduction**

Isosorbide dinitrate (ISDN) is an organic nitrate vasodilator which is extensively applied for the treatment of acute angina pectoris in humans [1]. The drug is administered orally in form of tablets and sustained-release preparation, sublingually, percutaneously as ointment and plaster on the skin or in i.v. dosage forms. Due to an extensive first-pass metabolism a large proportion of an orally administered dose of ISDN is metabolised to 2-isosorbide mononitrate (2ISMN) and 5-isosorbide mononitrate (5ISMN) which are considered pharmacologically active. Dynamic as well as therapeutic effects of ISDN are attributed largely to these metabolites [2 - 7]. A rapid metabolism of ISDN is effected by the enzyme glutathione S-transferase [8]. After the oral administration of ISDN in the range of 5 - 100 mg sustained release formulations, plasma concentrations of ng/ml are reached for ISDN and its two metabolites within few hours [9 - 16].

ISDN and its two metabolites possess good electron capturing properties. Williams and Murray applied for the first time an EC detector for the analysis of traces of ethylene glycol dinitrate and nitroglycerine in blood and urine [17]. Later an ECD was used for ISDN determination in blood [18]. A flame ionisation detector is insensitive for the detection of ISDN and metabolites in body fluids [19]. In recent years a number of gas-chromatographic methods with packed columns [9 - 25] and capillary columns [16, 26 - 28] have been reported for the determination of ISDN and its two metabolites 2ISMN and 5ISMN. TLC and HPLC have also been used for ISDN estimation [29, 30]. Packed columns in GC are generally unsuitable for the analysis of large numbers of plasma samples, especially when all the three components, ISDN, 2ISMN and 5ISMN, have to be detected simultaneously. They lack in this respect the required selectivity due to interfering peaks from plasma components and desired sensitivity. Capillary columns are characterised with specificity, good selectivity and high sensitivity. The determination of the three components could be generally attained simultaneously with little difficulty.

Among the number of methods reported in literature some are highly sensitive and accurate but require laborious and large numbers of time-consuming clean-up steps [21]
and if less time-consuming they are characterised with insensitivity [22] and poor reproducibility [11]. Single components such as ISDN [12, 23] or mononitrites [24] have been measured with high precision.

In the present study a simple extraction method is worked out for the simultaneous determination of ISDN, 2ISMN and 5ISMN in body fluids like plasma and saliva. The blood samples are taken after an oral administration of ISDN sustained-release tablets containing 80 and 120 mg drug each. Gas-liquid chromatography with different packed and fused silica capillary columns was used for the estimation of ISDN and its two major metabolites with an EC detector. The present method worked out is expedient, sensitive and reproducible for routinely handling a large number of samples for the simultaneous estimation of the three components.

Materials and Methods

All chemicals and solvents were of analytical grade and high purity. The solvents ethyl acetate, toluene and n-hexane were of “residue analysis” grade. Isosorbide dinitrate, 2-isosorbide mononitrate and 5-isosorbide mononitrate were obtained through the courtesy of Sanofi Schwarz Company, Monheim, FRG. All glassware, gc glass columns, centrifuge glass tubes and glass injection liners for fused silica capillary column were first cleaned with water, rinsed with methanol and then silanized thoroughly. The silanising solution consisted of 1% v/v each of hexamethyldisilazane, trimethylchlorosilane and pyridine in toluene. The glassware were kept immersed in this solution for 30 min and then rinsed well with methanol. The separation and determination of ISDN was performed on a Perkin-Elmer model F 22 gas chromatograph equipped with a 1-mCi nickel-63 ECD (wrapped in a 0.5 gm thick nickel foil) in the pulse mode. A Perkin-Elmer recorder 123 and a Hewlett-Packard Laboratory Data System 3352 C with a A/D convertor were connected to the gas chromatograph.

Chromatographic Columns

1 and 2 m x 2.4 mm i.d. coiled glass columns, silanised as described above were packed with 3% and 5% QF 1 on Gas Chrom Q, 100 – 120 mesh. The filled columns were flushed with carrier gas overnight. Argon with 5% methane was purified by passing through a Oxisorp filter (Messer Griesheim, FRG) and used throughout this study (for packed and capillary columns) as carrier gas. The carrier gas flow-rate was between 60-70 ml/min and the make-up gas for ECD was 70 ml/min. The temperature for 5 min. The mixture was then centrifuged for 15 min at 4,000 rpm. 0.500 ml of the clear supernatant liquid was withdrawn and gently evaporated to dryness with a stream of purified nitrogen. The residue was taken in 50 – 100 µl of ethyl acetate and then the centrifuge tube was put for a few seconds into an ultrasonic bath. 2 µl of this solution were injected into the gas chromatograph. The amounts of ISDN, 2ISMN and 5ISMN were obtained by comparing with the peak areas of the external standards in about the same concentration range.

Preparation of External Standards

1.00 mg/ml stock solutions of ISDN, 2ISMN and 5ISMN were prepared in ethyl acetate. They are stable for weeks when stored cool and in darkness. These solutions were further diluted with the solvent in the nanogram range. 0.500 ml of the blank plasma was taken in a centrifuge tube, aliquot amounts of all three nitrates were added and then the mixture was deproteinized with 100 mg ammonium sulfate. The solution was mixed thoroughly in a Vortex mixer and then extracted with 1 ml ethyl acetate as the plasma samples. Fresh standards were extracted every day with corresponding blank plasmas for each new sample series.

Sample Preparation with an Internal Standard

In another series of experiments α-HCH was used as an internal standard. Different compounds such as isomannide dinitrate, isooioidate dinitrate, nitroglycerine and dinitrochlorobenzene are reported in literature as internal standards in ISDN determinations [11, 12, 15, 23, 26, 28]. After testing