acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid, conjugate of **1** with L-methionine (**AP5**), 6(α-amino-γ-methyl thio butyrionamido) phenyl amino acetyl 3,3 dimethyl-7-oxo-4-thia-1-aza-bicyclo (3,2,0) heptane-2-carboxylic acid. Their physical characteristics are reported in the Table.

The partition coefficient of the synthesized compounds was determined between octan-1-ol and phosphate buffer (pH 7.4) [5]. The plasma protein binding of the compounds synthesized was determined by the equilibrium dialysis method reported by Vanderbelt using cellophane membrane and saline phosphate buffer (pH 7.4) [6]. The rate of hydrolysis of the compounds was determined at 37 ± 0.5 °C in simulated intestinal fluid and simulated intestinal fluid +10% plasma [7]. The James and Norman in situ rat gut technique was used to perform the absorption studies [8]. To estimate antibacterial activity of **AP1**, **AP2**, **AP3**, **AP4**, **AP5** and **1** the minimum inhibitory concentration (MIC) against *S. typhi*, *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtillis* was determined [9].

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A validated gas chromatographic method for the determination of isosorbide-5-mononitrate in human plasma and its application to pharmacokinetic studies

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Isosorbide-5-mononitrate (ISMN), the main pharmacologically active metabolite of isosorbide dinitrate (ISDN), is a vasodilator agent used in the prophylaxis and treatment of angina pectoris. ISMN, unlike ISDN, does not undergo first-pass metabolism in the liver, thus its absolute bioavailability is almost 100% [1, 2]. The ISDN concentration data show a considerable variability, as expected for a drug subject to first-pass metabolism [3]. This prompts the analysis of ISMN plasma concentrations in order to study the bioequivalence of ISDN formulations.

In recent years, a number of different GC methods coupled with electron-capture detection or mass spectrometry have been employed for the determination of ISMN in human plasma [4–8]. However, either these methods were not sufficiently sensitive or require expensive instruments or sample preparation techniques. The study of pharmacokinetic parameters in humans requires a sensitive and reliable analytical method. The typical C_{max} values of ISMN after a sublingual dose of 5 mg of ISDN are approximately 35 ng/ml [9]. Therefore, the objective of this study was to develop and validate a capillary GC method suitable for the quantitation of ISMN in real human plasma samples obtained in bioequivalence and pharmacokinetic studies, which require high sensitivity and selectivity.

Typical chromatograms obtained with a drug-free plasma and a plasma sample containing 19.5 ng/ml of ISMN obtained from a subject 12 h post-administration, after a single sublingual dose of 5 mg ISDN show no peaks, due to endogenous plasma components or ISDN, interfering with ISMN or internal standard. The retention times of ISMN and internal standard were 2.63 and 4.27 min, respectively. The total run time for an assay was approximately 5 min. The assay was validated over the concentration range 2.5 to 50 ng/ml ISMN. Data were obtained through linear regression analysis of peak height ratios versus concentrations of added ISMN. A weighting factor of 1/concentration was employed. The method was linear over the concentration range 2.5 to 50 ng/ml using a 0.5-ml plasma sample. A typical calibration curve had the regression equation of $y = 0.1626 + 0.1918x$ ($r = 0.9994$). Minimum quantitation limit was 2.5 ng/ml.

Inter-run assay precision assessed as the coefficient of variation values (CV%) was found to be 5.7%, 4.4%, and 4.2% for samples containing 10, 20, and 30 ng/ml, respectively. Inter-run accuracy, assessed as the relative error, was between 98.8% to 101.0%. Recovery was based on direct comparison of peak heights. The mean recoveries for ISMN were between $96.4\% \pm 6.5\%$ to $105.0\% \pm 3.1\%$. Mean recovery of internal standard was $100.9\% \pm 3.8\%$. Slightly higher than 100% recoveries are most likely due to partial evaporation of acetonitrile during sample preparation. System reproducibility expressed as the coefficient of variation values (CV%) and based on absolute peak height, was 8.4% and 6.8% for ISMN and 9.6% for internal standard.

In conclusion, a rapid, specific, sensitive, precise and accurate GC method with electron-capture detection for the

determination of ISMN in human plasma was developed and validated. The method was found to be suitable for the analysis of plasma samples obtained from 20 volunteers in the conduct of bioequivalence study of two sublingual 5-mg tablet formulations of ISDN [9].

Experimental

1. Chemicals and reagents

ISMN was purchased from European Pharmacopoeia (Strasbourg, France). Isomannide dinitrate, used as internal standard, was supplied from Elpen (Athens, Greece). Acetonitrile and ethyl acetate, both of HPLC grade, were purchased from J. T. Baker (Deventer, Netherlands). Isooctane for chromatography was obtained from Merck (Darmstadt, Germany). All other chemicals and solvents used were of analytical grade and water was milli-Q grade.

2. Chromatographic conditions

The GC system consisted of a Varian 3400 gas chromatograph coupled to a Varian electron-capture detector, a Varian 8035 autosampler (Walnut Creek, CA, USA), and a Hewlett-Packard HP3394A integrator (Avondale, PA, USA). Separation was performed on a 15 m × 0.53 mm DB-5 capillary column with a film thickness of 1.5 μm (J & W Scientific, Folsom, CA, USA). The injector temperature was 200 °C and the electron-capture detector was set at 220 °C with argon/methane (95 : 5) at a flow rate of 20 ml/min. The initial column temperature was 130 °C and the temperature was raised up to 145 °C at a rate of 5 °C/min, hold for 3 min.

3. Calibration curves and sample extraction

Working standard solutions of ISMN containing 25, 50, 100, 200, 350, and 500 ng/ml and of internal standard containing 4 μg/ml were prepared daily in acetonitrile/water (50 : 50, v/v). 50 μl of these working ISMN solutions and 50 μl of the working internal standard solution were added to 0.5 ml drug-free plasma to prepare the calibration standard samples containing 2.5, 5, 10, 20, 35, and 50 ng/ml of ISMN. Following addition of 1.5 ml of ethyl acetate, the samples were vortexed for 1 min and then centrifuged at 2000 × g for 10 min. The organic phase was transferred into another tube and evaporated to dryness at 20 °C with the aid of a gentle stream of air. The residue was dissolved in 0.5 ml of acetonitrile, 1 ml of isooctane was added and after vortexing for 1 min the isooctane was evaporated and 0.5 ml acetonitrile was added and mixed. This solution was transferred to autosampler vials and 2 μl were injected into the column.

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Mixing of pharmaceutical powders in tablet manufacture

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Suitable mixing of solid, particulate ingredients (powders) in tablet manufacture is of paramount importance in order to ensure a high quality product having appropriate content uniformity, not only of the active drug substance but of all components in the formulation.

Mixing of tablet ingredients is almost always accompanied with sieving, the main purpose of the latter being elimination or reduction of aggregates and agglomerates in the raw materials since most ordinary mixing equipment is unable to decompose them during mixing. Most pharmaceutically active agents are fine or very fine powders that are more or less prone to aggregation/agglomeration. Several excipients possess this property as well, even direct compression materials.

It appears to be common practice to start a mixing operation by sieving the raw materials separately followed by mixing in a suitable mixer. This approach, however, must be considered to be highly questionable since materials that tend to aggregate/agglomerate will rapidly re-aggregate/agglomerate after sieving. By contrast, premixing of the ingredients followed by sieving and remixing will in most cases effectively prevent the presence of aggregates/agglomerates in the finished blend due to the simultaneous intermixing of all ingredients effected by this method.

The above considerations generally apply to "ordinary" materials, i.e. those consisting of very fine or fine particles (approx. 20–100(200) μm). Some substances, e.g. micronized (particle size less than approx. 20 μm), highly cohesive or coarse materials may demand special sieving/mixing/milling methods. Elaborate procedures may also be necessary with formulations containing ingredients in very small amounts (less than approx. 1%).

The following example is given to illustrate the importance of the positioning of the sieving step in mixing operations. In this case the goodness of mixing could simply be estimated visually thus eliminating uncertainties possibly arising from demixing in sampling or handling of samples and/or chemical analytical errors.

3,750 g pregelatinized starch, 250 g colloidal silicon dioxide and 400 g red iron oxide were sieved separately through a 0.5 mm screen (Comil) and mixed for 5 min in a 25 l intensive mixer (Gral). This is called the "separate mix".

2,125 g Hydroxypropyl methylcellulose, 3,750 g pregelatinized starch, 46,500 g lactose, 250 g colloidal silicon dioxide and 15,500 g microcrystalline cellulose were sieved separately through a 1.0 mm screen (Comil) and mixed with the previously mentioned separate mix in a 300 l tumbling mixer (GEI IBC) at 12 rpm for 17 min.

Visual inspection of the resulting blend revealed the presence of numerous white aggregates/agglomerates in the powder bed. Consequently, the blend was sieved through a 1.0 mm screen (Comil) followed by mixing at 12 rpm for 5 min in the tumbling mixer. No aggregates/agglomerates could be detected visually in the resulting mixture.

These findings unambiguously demonstrate that sieving the ingredients separately prior to mixing did not prevent the presence of aggregates/agglomerates in the resulting