Comparison of the Absorption of Micronized (Daflon 500® mg) and Nonmicronized ^{14}C-Diosmin Tablets After Oral Administration to Healthy Volunteers by Accelerator Mass Spectrometry and Liquid Scintillation Counting

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ABSTRACT: Daflon 500® mg, is a micronized purified flavonoid fraction, containing 90% w/w diosmin and 10% w/w of flavonoids expressed as hesperidin, used clinically in the treatment of chronic venous insufficiency and hemorrhoidal disease. This study was designed to investigate the influence of particle size on the overall absorption of diosmin after oral administration of micronized (mean particle size = 1.79 μm, with 80% of particles having a size lower than 3.45 μm) and nonmicronized diosmin (mean particle size = 36.5 μm, with 80% of particles comprised between 19.9 and 159 μm). In a double blinded, cross-over study design, 500 mg tablets containing trace amounts (approximately 25 nCi) of ^{14}C-diosmin were administered to 12 healthy male volunteers as a single oral dose. Accelerator mass spectrometry and liquid scintillation counting were used for the measurement of ^{14}C-diosmin in urine and feces. Absorption of ^{14}C-diosmin from the gastrointestinal tract, measured by the urinary excretion of total radioactivity, was significantly improved with the micronized ($57.9 \pm 20.2\%$) compared with the nonmicronized material ($32.7 \pm 18.8\%$). Statistical comparison of the urinary excretion of the two pharmaceutical formulations showed this difference to be highly significant ($p = 0.0004$, analysis of variance). The overall excretion of the radiolabeled dose was 100% with mean ± SD of $109 \pm 23\%$ and $113 \pm 20\%$ for the micronized and nonmicronized forms, respectively. The results of this study show: 1. the impact of a reduction of particle size on the extent of absorption of diosmin, giving a pharmacokinetic explanation to the better clinical efficacy observed with the micronized formulation, and 2. the use of accelerator mass spectrometry in conjunction with liquid scintillation counting in measurement of bioavailability in a human cross-over study comparing two drug formulations containing trace amounts of radioactivity. J Pharm Sci 91:32–40, 2002

Keywords: micronization; diosmin; absorption; accelerator mass spectrometer; humans

INTRODUCTION

Oral absorption is a multifactorial process encompassing not only the *in vivo* dissolution of the drug, but also the physiological conditions in the gastrointestinal tract such as gastric emptying and the presence of bile acids, as well as the passage of the drug across the intestinal membrane.

The less a drug is absorbed, the more pharmacokinetic variation will occur from one subject to another, and the more it may be influenced by exogenous factors such as diet and dosing regimen.

For highly insoluble drugs, the limiting factor is more often the rate of dissolution than the

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passage of the intestinal barrier, and one way to better control the dissolution process is to reduce the size of the drug product particles. If the powder characteristics are adequate (structure, hardness, and chemical stability), micronization is a well-established technique to attain this objective.[1] Many *in vitro* dissolution and *in vivo* studies have validated this concept.

Diosmin, chemically defined as 7-{[6-O-(6-deoxy-a-L-mannopyranosyl)-β-D-glucopyranosyl] oxy}-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzo[b]pyran-4-one, is clinically used as a micronized formulation with improved pharmacodynamic and clinical activities in the treatment of chronic venous insufficiency,[2,3] and hemorrhoidal attacks.

The objective of this study was to confirm a previous study performed in animals[4] and document the impact of particle size on drug absorption by comparing two tablet formulations (micronized and nonmicronized) of a purified flavonoid fraction (S 5682) enriched with trace amounts (25 nCi) of ^{14}C-diosmin. These tablets were administered in a double blinded, cross-over study, to 12 healthy male volunteers as a single oral dose.

Accelerator mass spectrometry (AMS), which enables the efficient measurement of very small quantities of rare and long-lived isotopes such as ^{14}C, with high precision,[5–8] was chosen in combination with super low level liquid scintillation counting (LSC) for the assessment of the excretion balance. With AMS detection, several administrations to humans of a trace amount of radiolabeled compound can be used (1 to 500 nCi); indeed, the need for approval by radiopharmacy committees is no longer required.[9]

MATERIALS AND METHODS

Study Drugs and Labeled Compound

Purified flavonoid fraction (S 5682) batch 53879 was supplied by Technologie Servier (Orleans, France). ^{14}C-labeled diosmin (Fig. 1) was synthesized by Nycomed Amersham Plc. (Buckinghamshire, England). Specific activity was 2.07 GBq/mmol (56 mCi/mmol) with a purity of 98%.

Tablet Preparation

Preparation of the tablet formulations with the radiolabeled drug substance: The tablets were prepared according to a similar process and with the same formulation as that described for the core tablet in the submission file.

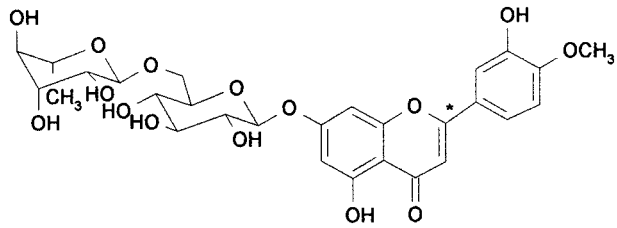

Figure 1. Chemical structure of diosmin and position of ^{14}C-label (*).

The radiolabeled drug substance was dissolved with an unlabeled drug substance in a 1 N sodium hydroxide solution. The drug substance was precipitated by the addition of a 1 N hydrochloric acid in the drug solution. The resulting precipitate was washed on a Nalgene 0.8 μm filter with purified water until a chloride residue of < 1000 ppm was obtained. This precipitate was further dried using a freeze dryer (Serail RPV2, Argenteuil, France). The dry product was sifted on a 315 μm mesh size screen and divided in two equal parts: the first part was considered as the nonmicronized product and the second part was micronized using a cylindrical air micronizer (CECA 2″, Paris, France) in order to obtain a particle size of < 2 μm. Two successive operations were necessary. The particle size was measured by laser diffraction (Malvern SB20, Orsay, France).

Tablets were prepared by wet granulation. The granulation was conducted with the drug substance and the binder (Cryogel; PB Gelatine, Vilvoorde, Belgium) using a high shear mixer (Moulinex BabyChef, Paris, France). The wet granule was dried at 50°C using a vacuum dryer (Bioblock, Illkirch, France) to obtain a residual humidity of less than 5% and then sieved using a 1-mm mesh size screen. The sieved granules were mixed with micro-crystalline cellulose (Avicel PH102; Seppic, Paris, France) using a Turbula Mixer (Prolabo, Paris, France) and lubricated with sodium starch glycollate (Primojel, Avebe, France), magnesium stearate (Akros, The Netherlands), and talc (Talc de Luzenac, Toulouse, France) using the same Turbula mixer. The lubricated granules were compressed using a reciprocating tabletting machine (Korsch EK-0, Berlin, Germany) and oblong punches and die. The targeted mass of the tablets was 650 mg.

Study Design

The study was a monocentric, double blinded, cross-over study in 12 healthy male volunteers (age 34.5 ± 9.8 years and weight 73.2 ± 7.2 kg) approved by an independent Medical Ethics Committee. Treatments were allocated by balanced randomization, without stratification. Each subject received a single oral dose of ^{14}C-micronized diosmin or ^{14}C-nonmicronized diosmin with a wash-out period of 14 days. The dose of S 5682 was 500 mg (containing approximately 25 nCi of ^{14}C-labeled drug) and was administered as a single tablet (Table 1). Subjects took neither food nor drink for approximately 12 h before the administration. The subjects ate a standardized meal 3 h and 9 h after drug intake. The subjects also drank at least 1.5 L of water or decaffeinated coffee per day.

Sampling Schedules

The dosing and sample collection were conducted by Simbec Research Limited (Merthyr Tydfil, UK).

Total voided urine was collected from all subjects into preweighed polypropylene containers before dosing (12–0 h) then at 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 h after dosing. After each collection, urine was kept refrigerated (+4°C) until the end of the collection period.

Total voided feces were collected from all subjects into preweighed polypropylene containers on day 1 before dosing and thereafter were pooled approximately every 24 h up to at most 168 h after dosing.

Analytical Methods

All submitted samples were prescreened for radioactive content by LSC.

LSC

Before LSC analysis performed with a scintillator Packard (Paugbours, UK) Tri-Carb TR/SL 2770, urine samples were thawed, vortex-mixed thoroughly, and then centrifuged for 5 min at 3500 rpm. Aliquots (0.5 mL) of urine samples were taken and added to Ultima Gold scintillant (4.5 mL) (Packard) in a single scintillation vial. A blank was prepared by adding 0.5 mL of high-pressure liquid chromatography (HPLC)-grade water to Ultima Gold scintillant (4.5 mL); this was used to provide a background sample. The vials were capped, shaken, left to cool and dark adapt in the LSC for 2 h, and windows set at 0.0–156 keV and background subtract.

Original feces samples were diluted at 50% with methanol and blended. Each fecal sample was analyzed in triplicate by taking approximately 500 mg of this mixture, oxidized using a Canberra Packard Model 307 Biological Sample Oxidizer. The oxidizer's gaseous sample was dispensed in Permafluor E + scintillant and collected in a single scintillation vial. Triplicate samples were allowed to cool and dark adapt in the LSC for at least 2 h before being counted for 30 min per sample. The acceptance criteria were established for these analyses and referred only to those samples having a cpm value of at least one and a half times background, i.e., > 5 cpm after background subtraction. The replicates for these samples had to have a coefficient of variation of 15% or less to be accepted. Samples that did not

Table 1. Description of the Study Formulations

Composition: Ingredients	Nonmicronized Formulation (mg)	Micronized Fomulation (mg)
Purified, flavonoic fraction enriched with 25 nCi ^{14}C-diosmin	500	500
Average moisture content	20	20
Gelatin	31	31
Magnesium stearate	4	4
Microcrystalline cellulose	62	62
Sodium starch glycollate	27	27
Talc	6	6
Mean particle size	36.5 μm	1.79 μm

initially fit within the acceptance criteria were recounted for 60 min to achieve lower coefficients of variation, whereas some samples had to be reanalyzed.

AMS

Total radioactivity in urine and feces samples was determined by AMS. The limit of detection was 0.001 to 0.01 dpm/mL. In order for samples to be analyzed by AMS, the carbon within the sample must first be converted to graphite via a two-step process of oxidation and reduction. In brief, samples were oxidized to CO_2 in the presence of copper oxide at 900°C and the resultant CO_2 was then reduced to filamentous graphite on cobalt, in the presence of zinc powder and titanium hydride at 500–550°C. In addition, because AMS provides an isotope ratio, i.e., $^{14}C/^{12}C$ and not an absolute value, it was essential to know the carbon content of the sample plus any added carrier. This was determined using a C, H, N analyzer or using known percentage carbon contents.

C, H, N Analysis

Analysis was performed on a C, H, N NA2100 Brewanalyser. Thirty microliters of each plasma and urine sample was adsorbed onto Chromosorb W in a tin capsule.

One tube of each of the fecal samples was emptied into a tared container and an equal weight of HPLC-grade methanol ± 0.1 g was added. In order of ascending activity (predetermined by LSC analysis), fecal samples were blended using an Ultra-Turrax blender. Thirty microliters of each of the blended fecal samples was adsorbed onto Chromosorb W in a preweighed tin capsule. Drying the samples in a furnace set to 90°C for 30 min evaporated off the methanol component of each sample.

For all urine and feces, tin capsules were closed, pressed, and loaded into an autosampler to be automatically inserted into the C, H, N analyzer.

A calibration curve was obtained using urea standards (100 mg/mL), and was used to determine the carbon content of each sample.

AMS Analyses

All samples were thawed before processing. One hundred fifty microliters of each urine sample was placed in clean, baked glass sample tubes together with prebaked copper oxide wire (50 ± 10 mg). One hundred microliters of carrier tributyrin (TBT) solution (20 mg/mL in methanol, containing 59.6 % carbon) was added to the sample tubes containing urine aliquots and the whole dried under vacuum using a Savant (Bosungstoke, UK) AES2010 Speed Vac. The final carbon amount for graphitization was approximately 2 mg carbon. Standards and controls—ANU sugar (5–7 mg) and synthetic graphite (2–3 mg) were placed in separate sample tubes containing copper oxide. One hundred microliters of tributyrin control solution (20 mg/mL solution in methanol) was also placed in separate sample tubes with copper oxide. All standards and controls were dried under vacuum as described above.

Five microliters of each fecal sample was placed in clean, baked glass sample tube together with prebaked copper oxide wire (50 ± 10 mg). The samples were concentrated to dryness under vacuum using a Savant AES2010 Speed Vac. One hundred microliters of carrier tributyrin solution (20 mg/mL in methanol, containing 59.6 % carbon) was added to the sample tubes and again dried under vacuum in the Speed Vac. The final carbon amount for graphitization was approximately 2 mg carbon. Obtaining the dry sample mass used for AMS analysis combined with the known percentage carbon content determined by C, H, N analysis was necessary to be able to ascertain the dpm per milligram of sample.

Fecal samples were also extracted providing fecal extract and fecal residue samples for AMS as follows. Approximately 10 g of fecal homogenate (diluted 1:1 with HPLC-grade methanol and blended) were centrifuged for 10 min at 2500 rpm. The supernatants were decanted into preweighed Falcon tube allocated to each sample. The fecal pellets were washed by adding approximately 10 mL of HPLC-grade methanol and vortex mixing for 2 min. The resuspended pellets were centrifuged again at 2500 rpm for 10 min. The supernatants were decanted into the same Falcon tube as the previous supernatant for each sample. To dry the pellets, tubes containing the residues were dried in the Speed Vac for 2 h with 80 min radiant cover on a high heat setting. After being dried, all pellets were reweighed, as were the supernatants.

C, H, N analysis was conducted on the fecal residues to determine the weight that would be required to provide approximately 2 mg of carbon for AMS analysis. For this process, 1–5 mg of each residue was weighed into a tared tin capsule, which was then closed and pressed before being inserted into the autosampler for automatic release into the C, H, N analyzer.

For AMS analysis, 4–5 mg of fecal residue was weighed into tared sample tubes containing copper oxide wire; tributyrin was not required. Fifty microliters of each of the fecal extracts was aliquoted onto copper oxide wire in sample tubes along with 100 μL of 20 mg/mL tributyrin solution.

Tributyrin was used for the carbon carrier because it has a relatively low ^{14}C content, high carbon content, low nitrogen content, low vapor pressure, and high solubility in alcohol and is nonhazardous.

Combustion (Oxidation)

The glass sample tube containing the dried sample and copper oxide was placed into a larger glass combustion tube, which was heat-sealed under vacuum and heated at 900°C for 2 h in a Carbolite furnace. After combustion, the tubes were allowed to cool slowly to ambient temperature.

Graphitization (Reduction)

The pointed end of the larger combustion tube was placed in a plastic Y-manifold to the other arm of which was attached to a glass graphitization tube.

The latter tube contained zinc powder and titanium hydride mix in the ratio 2.5:3 w/w (120–200 mg). Inside the graphitization tube was a culture tube containing cobalt powder (6.5 ± 1.5 mg). The combustion tube was dipped into an isopropanol/dry ice bath and the graphitization tube into a bath of liquid nitrogen. The whole system was placed under vacuum and the carbon dioxide formed from the oxidized sample cryogenically transferred to the graphitization tube, after breaking the combustion tube tip. Once transferred, the graphitization tube was heat-sealed under vacuum and placed in a furnace and heated at 500°C for 4 h, followed by a further 6 h heating at 550°C before slow cooling to ambient temperature.

Once the graphitization process had been completed, the graphite was left in the sealed graphitization tube until ready to be packed into a cathode. To pack the cathode, the graphitization tube was opened and the culture tube containing the graphite adsorbed onto the cobalt catalyst removed. The cobalt/graphite was carefully tipped out into an aluminum cathode and compressed into place at 170–200 psi in a Parr Pellet Press to form a tablet of graphite within the cathode. When required for analysis, the cathodes were placed into a 134-position AMS sample wheel and the sample wheel was stored under vacuum.

The sample wheel in which the graphite-containing cathodes were placed, was inserted into the ion source of the AMS instrument (NEC 15SDH-2 Pelletron AMS system; National Electrostatics Corp., Middleton, WI). The multi-cathode negative ion source (MC-SNICS) generated a cesium (Cs^+) ion beam that was accelerated onto the graphite surface. The resulting negative carbon ion beam contained $^{12}C^-$, $^{13}C^-$, and $^{14}C^-$ and other ions such as $^{16}O^-$. The isobar $^{14}N^-$ is unstable and therefore cannot interfere with the ^{14}C measurement. The carbon ion beam was preaccelerated, passed through a spherical electrostatic analyzer, and then progressed toward the injection magnet. Output of $^{12}C^-$ was typically 1–100 μA. The magnet was set to inject $^{12}C^-$ (l50 μs), $^{13}C^-$ (600 μs), and $^{14}C^-$ (0.1 s) ions sequentially at normally 68 keV; one combined measurement on each isotope in turn corresponded to one cycle. The carbon ion beam was accelerated toward the positive center terminal of the tandem Pelletron accelerator through an Einzel lens. The terminal voltage used for this series of analyses was 4.5 MV with particle energy of approximately 22.5 MeV. At the central terminal, electrons were stripped from the carbon atom to yield positively charged carbon ions ($^{12,13,14}C^{+1\ \text{to}\ +6}$). C^{4+} ions were selected for measurement, because these were the most abundant at this energy. These ions were accelerated away from the center terminal and onward toward the electrostatic quadrupole triplet and analyzing magnet.

Immediately past the postanalyzing magnet, $^{12}C^{4+}$ and $^{13}C^{4+}$ ions were measured as an ion current in offset Faraday cups. $^{14}C^{4+}$ ions were passed down the high energy beam line, through an electrostatic quadrupole doublet and a cylindrical electrostatic analyzer. From there, the ions entered a gas ionization detector where they were collected on anodes (four in total) which measured the energy loss and total energy of each ion. Other interfering non-$^{14}C^{4+}$ ions were generally prevented from entering the gas ionization detector by the combinations of electrostatic analyzers, magnets, slits, and charge state separation. Vacuum pressures of approximately 10^{-9} Torr were maintained in the beam line and 10^{-6} Torr in the ion source. Ion transmission through the instrument was between 30–60%. The burn-in time for each graphite sample was set to 600

cycles (60.4 s) and sample analysis consisted of 2500 cycles (251.75 s) for urine samples, 2000 cycles (201.4 s) for analysis of fecal homogenates, and 1000 cycles (100.7 s) for the fecal residues and fecal extracts.

Pharmacokinetic Analyses

The amount of radioactivity in each sample of urine and feces, calculated from the concentration of radioactivity together with the weight or volume of that sample, was expressed as a percentage of the administered radioactive dose. From the urinary and fecal data, the following parameters were calculated: Ae—cumulative amount of total radioactivity excreted in the urine (0–168 h); and AeF—cumulative amount of total radioactivity excreted in feces (0–168 h).

Statistical Analysis

The objective of the statistical analysis was to compare the cumulative urinary excretion data obtained after single oral administration of the two different formulations administered, a nonmicronized and a micronized form in the same subject with a cross-over design. The statistical analysis consisted of an analysis of variance, and the test was performed at the 5% level of significance. The statistical analyses were performed using Microsoft EXCEL 97 software and the WinNonlin Professional software (version 1.5).

RESULTS AND DISCUSSION

Drug Formulation

Manufacture of the tablets was performed to match the specification of the final formulation used in the clinic. The presence of radioactive material, negligible in terms of the individual dose given to the healthy volunteers, still implies formulation on a smaller-scale machine with adapted safety conditions during the manufacturing process.

The tablets were sampled and tested for purity (radiochemical and nonlabeled drug purity) granulometry, and homogeneity. Radiochemical purity was 98.3% and the amount of radioactive material was 24.2 nCi (coefficient of variation = 3.9%) and 22.4 nCi (coefficient of variation = 6.5 %) per tablet in micronized and nonmicronized formulations, respectively. The mean particle size was 1.79 μm and 36.5 μm in micronized and nonmicronized formulations, with 80% of particles having a size lower than 3.45 μm for the micronized formulation, and 80% of particles comprising between 19.9 and 159 μm for the nonmicronized formulation, respectively.

As a result of the very low solubility of diosmin in water and the necessary presence of high quantities of sodium hydroxide, no dissolution profiles were performed.

Excretion Balance of Total Radioactivity

Initial LSC were performed for urine and fecal samples to avoid saturation of the AMS detector with high levels of radioactivity.

Urine

After the administration of the micronized formulation, urinary excretion accounted for $57.9 \pm 20.2\%$ of the dose as compared with $32.7 \pm 18.8\%$ (Table 2) with the nonmicronized tablets. As urinary data are indicative of the minimum absorbed fraction, there is a clear confirmation that reduction of the particle size using a micronization process improves the overall absorption of diosmin (Figs. 2 and 3). Statistical comparison of the urinary excretion of the two pharmaceutical formulations showed this difference to be highly significant ($p = 0.0004$, analysis of variance), with an increase of absorption close to 80% between both formulations.

This data are in good agreement with previous data (Servier internal reports) where, after administration of 500 mg of nonmicronized diosmin as a suspension to 12 healthy young male volunteers, 3.8 to 29.8% of the dose was recovered in urine.

In addition, urinary elimination was predominant in the first 24 h samples and more marked with the micronized ($31.1 \pm 11.1\%$) as compared with the nonmicronized formulation ($15.9 \pm 6.0\%$) (Table 2 Fig. 2). Identical results have been obtained in humans where reduction of the particle size of some drugs improved the bioavailability of poorly soluble drugs.[1]

Feces

As a standard procedure, feces were diluted in methanol to obtain a homogeneous preparation before radioactivity determinations.

Table 2. Individual and Mean Cumulative Excretion of Total Radioactivity in Urine and Feces After a Single Oral Administration of 500 mg of Micronized S 5682 Enriched With Micronized ^{14}C-Diosmin (Part A) and 500 mg of nonmicronized S 5682 Enriched With Nonmicronized ^{14}C-Diosmin (Part B)[a]

	Subject No.													
Period (h)	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
A: Micronized S 5682 enriched with micronized ^{14}C-diosmin ^{14}C-S 5682														
Urine														
0–24	30.9	51.8	33.8	21.9	30.1	33.0	31.6	39.1	42.1	24.8	27.4	7.03	31.1	11.1
0–48	36.7	60.7	73.4	38.6	44.4	50.5	35.5	56.5	71.6	32.6	42.7	51.1	49.5	13.7
0–72	37.2	64.1	89.6	39.1	45.1	53.9	36.2	56.8	78.9	33.8	47.9	70.9	54.4	18.2
Ae	37.9	65.2	93.1	40.3	46.2	55.0	37.2	70.2	83.9	35.0	50.4	80.1	57.9	20.2
Feces														
0–24	22.2	0	0	0	0	0	0	5.95	0	6.95	0	0	2.92	6.56
0–48	63.6	9.76	29.4	51.5	57.6	0	81.4	43.1	0	62.6	40.1	0	36.6	28.5
0–72	63.6	15.9	37.1	51.5	57.6	8.63	81.4	68.5	0	78.3	62.9	0	43.8	30.3
AeF	63.6	15.9	37.1	51.5	57.6	8.63	81.4	68.5	23.2	78.3	62.9	62.7	50.9	24.2
Cumul. amount	102	81.1	130	91.7	104	63.6	119	139	107	113	113	143	109	23.0
B: Nonmicronized S 5682 enriched with nonmicronized ^{14}C-diosmin														
Urine														
0–24	12.9	20.7	13.6	14.1	18.2	14.3	9.62	24.2	29.1	11.1	12.6	9.97	15.9	6.02
0–48	16.9	28.4	30.9	19.6	21.3	41.9	10.7	32.5	53.4	22.0	22.3	16.0	26.3	12.0
0–72	17.8	29.9	38.5	20.3	21.8	46.1	10.9	33.7	55.2	23.1	27.2	18.1	28.5	12.9
Ae	18.8	31.0	42.5	21.4	22.9	47.4	11.8	34.9	83.3	24.3	29.5	24.3	32.7	18.8
Feces														
0–24	61.8	0	0	0	16.9	0	22.8	21.2	0	41.3	0	0	13.7	20.3
0–48	102	40.7	0	47.7	104	24.1	91.9	96.6	11.0	108	28.9	0	54.5	43.0
0–72	102	70.3	40.8	65.7	104	24.1	91.9	96.6	20.2	108	46.9	22.1	66.0	34.2
AeF	102	70.3	77.5	65.7	104	24.1	91.9	96.6	50.0	108	75.5	98.8	80.3	25.1
Cumul. Amount	121	101	120	87.1	127	71.5	104	132	133	132	105	123	113	19.5

[a]The results are expressed as % of administered dose. AMS was used for urine radioactivity determinations and LSC for fecal measurements; Ae = cumulative amount of total radioactivity excreted in the urine (0–168 h); AeF = cumulative amount of total radioactivity excreted in feces (0–168 h); Cumul. = cumulative; SD = standard deviation.

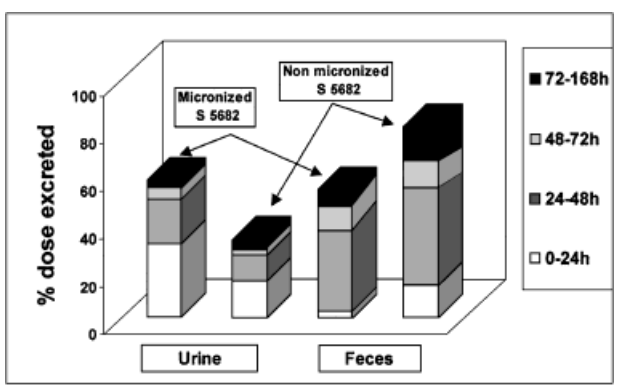

Figure 2. Mean ($n = 12$) cumulative urine and feces excretion of ^{14}C-radioactivity after single oral dose administration of 500 mg of micronized S 5682 enriched with micronized ^{14}C-diosmin (micro) versus 500 mg nonmicronized S 5682 enriched with nonmicronized ^{14}C-diosmin (nonmicro). The results are expressed as % of administered dose.

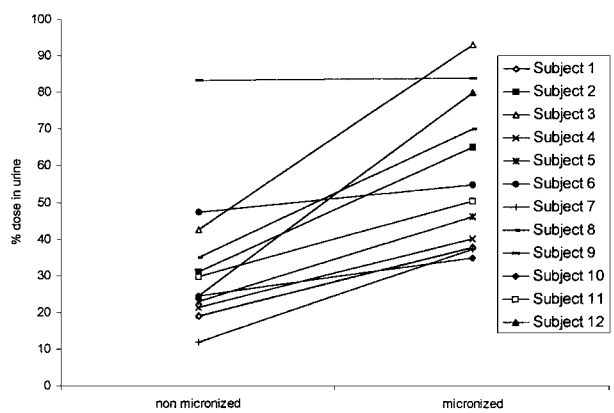

Figure 3. Comparison of urinary excretion of total radioactivity in healthy volunteers after oral administration of 500 mg of micronized S 5682 enriched with micronized ^{14}C-diosmin versus 500 mg of nonmicronized S 5682 enriched with nonmicronized ^{14}C-diosmin. The results are expressed as % of administered dose.

Preliminary LSC analyses revealed that levels of radioactivity were too high in the early fecal samples for AMS analysis. The supplied samples were therefore further diluted with methanol before analyses by AMS. The results were too variable and not consistent with those measured by LSC even after extraction of feces with methanol, separation of the pellet from the extraction solvent, and AMS analyses of both residues.

This lack of homogeneity of the diluted sample was a consequence of the very low solubility of diosmin, known to be practically insoluble in water and poorly soluble in alcohol. However, reanalysis of the initial methanolic fecal suspension by LSC gave reproducible results, and despite the lack of sensitivity of this method for late time point samples, LSC results were retained for the calculation of the overall excretion balance.

When combining both urinary AMS and LSC fecal data, the excretion balance for both formulations was complete with a total recovery of $109 \pm 23\%$ for the micronized and $113 \pm 20\%$ for the nonmicronized formulation, respectively.

Application of AMS to Pharmacokinetic Studies

Classical human pharmacokinetic studies using decay counting methods commonly use 50 to 300 μCi of ^{14}C-labeled drug,[10,11] but these doses are generally not compatible with clinical studies using cross-over design; indeed, the washout period should be far too long to recover between two administrations. With the use of AMS, 1 to 500 nCi (1 microSievert or below)[12] are sufficient for quantification. The need for approval by radiopharmacy committees is no longer required at such dose levels which are by far below the dose of radioactivity authorized in humans allowing several administrations of radiolabeled drug. This method is therefore of use to study drugs with high intersubject variability (e.g., insoluble drugs for which absorption processes are influenced by many physicochemical and physiological factors) where repeated administrations of radiolabeled drug are required.

If AMS has proven its usefulness and precision in following radioactivity in urine samples, radioactivity measurements of fecal samples were too variable to be used in this study owing to the inability to obtain a diluted homogeneous sample. Fecal radioactivity levels were often > 20 dpm/g, the maximum radioactivity that is permitted to put into the AMS instrument. The combination of a low solubility drug and a high sensitivity detection technique such as AMS implies a strict control of the homogeneity of the preparations because of the high dilution factors applied. Nevertheless, we have demonstrated here for the first time the power of using AMS for formulation studies where two or more formulations can be compared in the same group of subjects. The use of a cross-over design removes some of the interindividual variability between subjects because the same person acts as their own control.

CONCLUSIONS

The data reported herein demonstrate that reduction of particle size obtained by a micronization process improves the extent of absorption of diosmin and/or its metabolites, giving a pharmacokinetic explanation to the better clinical efficacy observed with the micronized formulation.

Increasing the absorbed fraction will also lead to lower variability from one patient to another, and less influence of exogenous factors such as diet and dosing regimen.

The results of this study indicate that the use of AMS has a great potential in the field of pharmaceutical formulation development, enabling the administration, as single or repeated dosing, of very low quantities of radioactivity, e.g., low nCi doses of ^{14}C in classical clinical studies.

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