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Short communication

Matrix solid-phase dispersion technique for the determination of a new antiallergic drug, bilastine, in rat faeces☆

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Abstract

A matrix solid-phase dispersion (MSPD) procedure for the isolation and HPLC determination of a new antiallergic agent, bilastine, in rat faeces is presented. The effect on recovery of empirical variables such as nature, pH and volume of the washing and elution liquids and nature of the adsorbent has been tested. The best recoveries were attained using an octadecylsilyl sorbent, 10 ml of a 0.1 *M* $NaHCO_3$–Na_2CO_3 aqueous buffer of pH 10.0 as washing solvent and 10 ml of methanol as elution solvent. The extracts were evaporated to dryness and reconstituted in mobile phase before their injection into a HPLC system, equipped with a Discovery RP-amide C_{16} column and a fluorescence detector. The method allows one to reach recoveries of 95.0% within the concentration range 0.05–10 μg/g, with within-day repeatabilities of less than 5% and between-day repeatabilities of less than 9% within this range. This method has been successfully applied to the excretion studies of bilastine in the rat.

Keywords: Matrix solid-phase dispersion; Bilastine

1. Introduction

Methods for the isolation of compounds from solid biological matrices, such as tissues or faeces, often begin with a process designed to disrupt the general architecture of the sample (mechanical blender or grinding with abrasives). Sonication, addition of chemical reagents, surfactants or enzymes can help to obtain an appropriate homogenate. Liquid–liquid extraction, solid-phase extraction, centrifugation, counter-current extraction and/or column chromatography are used to clean up these homogenates before their introduction into chromatographic systems. These clean-up procedures are usually very laborious and time and reagent consuming. Often the high degree of sample manipulation leads to poor recoveries.

More efficient techniques to reduce the number of steps in sample clean-up are needed. In this sense, matrix solid-phase dispersion (MSPD) can be an aid [1–6]. This technique involves grinding of the sample with silica solid sorbents (octadecylsilyl or others), packaging of the mixture into a syringe

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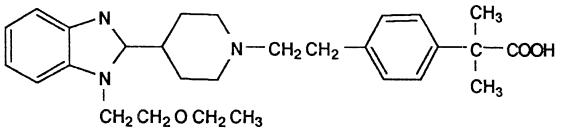

Fig. 1. Structure of bilastine.

barrel column, washing with suitable solvents and elution and collection of the analytes with an appropriate solvent.

MSPD has been used for different analytes and matrices: vitamins in infant formula and medical foods [7,8]; antibiotics and veterinary residues in meat, milk and cheese [9–12]; pesticide residues in fruits, vegetables, milk and other foods [3,4,13–16]; pesticides, poychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and surfactants in fish [2,17]; pesticide residues, PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in milk, yoghurt and butter [18,19]; and pesticide residues in human serum [20]. To our knowledge no application of MSPD to faeces has been made until now. Faeces are usually treated, after an initial homogenisation, by extraction with a solvent followed by additional clean-up by liquid–liquid or solid-phase extraction techniques [21–24].

In this work an MSPD procedure for the isolation and later liquid chromatographic determination of a new antiallergic agent, bilastine (Fig. 1), in rat faeces is presented. The effect on recovery of empirical variables such as nature, pH and volume of the washing and elution solvents and nature of the adsorbent has been tested. This method has been successfully applied to the excretion studies of bilastine in rat.

2. Experimental

2.1. Chemicals and reagents

Bilastine (FAES, Leioa, Spain) had a chromatographic purity of 97.8%. Methanol, acetonitrile and acetone were of HPLC quality from Romil (Harvehill, UK) and Ultrapure water was obtained with a Milli-Q system from Millipore (Bedford, MA, USA). All other chemicals were of analytical grade: sodium acetate, disodium hydrogenphosphate, sodium hydrogencarbonate and sodium carbonate from Fluka (Buchs, Switzerland); acetic acid, sodium dihydrogenphosphate and sodium chloride from Merck (Darmstadt. Germany).

Stock standard solutions of bilastine 1000 μg/ml were prepared in methanol and stored at 4°C in the dark. These stock solutions were stable under these storage conditions for almost 3 months. Working solutions were prepared daily by dilution from the stock solution. Aqueous buffers were prepared for use as washing solvents at two concentration levels, 0.1 and 0.01 *M*. Aqueous solutions of sodium chloride 9‰ (m/v) were used for the homogenisation of the faeces.

2.2. Equipment

Faeces samples were homogenised using a glass homogeniser of adequate size (Refs. 5315 or 5318) from Afora (Barcelona, Spain).

The materials for the MSPD processes were: syringe plungers (inserters), empty polypropylene syringe barrels (15 ml) and polypropylene filter disks (20 μm) from IST (Hengoed, Mid-Glamorgan, UK). Isolute C_{18} (40–70 μm, part No. 9370) and C_{18} EC (40–70 μm, part No. 9371) adsorbents from IST and Florisil (60–100 mesh) from Fluka. Liquids were passed through the packed syringe barrels using a multiple solid-phase extraction (SPE) device (Visiprep Vacuum Manifold) from Supelco (Bellefonte, CA, USA). Another Visiprep Vacuum Manifold was used for the evaporation of the extracts.

The liquid chromatograph employed was from Hewlett-Packard (Palo Alto, CA, USA) and consisted of a quaternary pump HP1100, an automatic injector HP1100 and a fluorescence detector HP1100.

2.3. Sample preparation

Spiked blank samples were prepared mixing into a glass homogeniser a weighed mass of untreated rat faeces (e.g., 1 g), the appropriate amount of a

standard dilution of bilastine and an adequate quantity of NaCl 9‰ (e.g., 2 ml). Faeces samples from rats treated with bilastine were homogenised in the same way using NaCl 9‰ in an approximate relation 1:2 (m/v) depending on the moisture of each sample.

An aliquot of the homogenate (less than 0.3 g) was mixed and gently ground with 0.8 g of MSPD adsorbent into a glass mortar with a pestle until a nearly homogeneous blend of sample on the adsorbent was obtained (1–2 min). For better homogenisation it is necessary to scrape the sides of the mortar and pestle with the aid of a spatula before grinding again. The blend is transferred using the spatula to a syringe barrel that contains a filter disk in the bottom and 0.8 g of MSPD adsorbent. Another portion of 0.4 g of adsorbent is added to the mortar, ground and transferred to the syringe barrel, in order to remove the last remains of the sample. The column head is covered with another filter disk and the contents are compressed using the syringe plunger. Different liquids can be passed through the syringe barrel in order to wash the sample or elute the analyte. The overall time spent for the preparation of each sample (homogenisation+MSPD) was about 40 min.

2.4. Column liquid chromatography

Extracts obtained from the MSPD processes were evaporated to dryness, reconstituted with 1 ml of mobile phase, filtered trough a membrane of 0.45 μm and 20-μl injected into the liquid chromatograph. The column was a Discovery RP-amide C_{16} (150×4.6 mm) 5 μm, protected with a Discovery RP-amide C_{16} pre-column (20×4 mm) 5 μm, both from Supelco. The elution was performed isocratically using a mobile phase of acetonitrile–0.02 *M* NaH_2PO_4 (pH 4.0 adjusted with 0.75 *M* H_3PO_4) (25:75, v/v) delivered at a flow-rate of 0.8 ml/min at room temperature. After the elution of the analyte (27 min) a washing step of the column is needed, increasing the acetonitrile percentage up to 75% in 5 min, maintaining this composition for 5 min and recovering the initial composition of mobile phase in other 5 min. The detection was carried out at excitation and emission wavelengths of 250 and 310 nm, respectively.

3. Results and discussion

3.1. MSPD optimisation

Two MSPD adsorbent materials, C_{18} and C_{18} EC (with end-capping), were tested using blank faeces spiked at a concentration level of 10 μg/g. Moreover, the effect of an additional layer of Florisil (1 g) below the MSPD material was assayed. Different solvents were successively passed through the syringe barrel and collected with these four possible configurations and in order of decreasing polarity (aqueous buffer, methanol, acetonitrile and acetone). The use of Florisil or buffer of pH 4 greatly reduced the recoveries of bilastine in all cases. Moreover, losses of bilastine in the aqueous fraction were observed when C_{18} EC adsorbent and buffers of pH 7 or 10 were used. Then the best conditions were C_{18} adsorbent and buffer of pH 7 or 10; in both cases no losses of analyte were observed in the aqueous fraction and bilastine was nearly completely concentrated in the methanol fraction.

In order to check the possibility of introducing a more efficient washing step mixtures of methanol–buffer, pH 10 or small volumes of methanol were tested, but losses in bilastine were observed with the first fractions assayed in each case (methanol–buffer, 20:80, v/v, or 4 ml of methanol). Additionally, no differences in recoveries were found when 0.1 or 0.01 *M* buffer concentrations were used. Thus, the optimal conditions finally selected for the MSPD process were C_{18} as adsorbent, 10 ml of 0.1 *M* buffer of pH 10 for washing and 10 ml of pure methanol for elution of bilastine.

3.2. Validation and application to excretion studies samples

Typical chromatograms for blank and spiked rat faeces are shown in Fig. 2. Stability of bilastine along the analytical process was tested comparing the results of a spiked sample analysed quickly, without dead times, and the same sample analysed over a long period of time (5 days), stopping in some critical moments of the analytical process: when the syringe barrel is packed (1 day), the extract of methanol (closed during 3 days at room temperature) and in the injector vial in mobile phase (closed

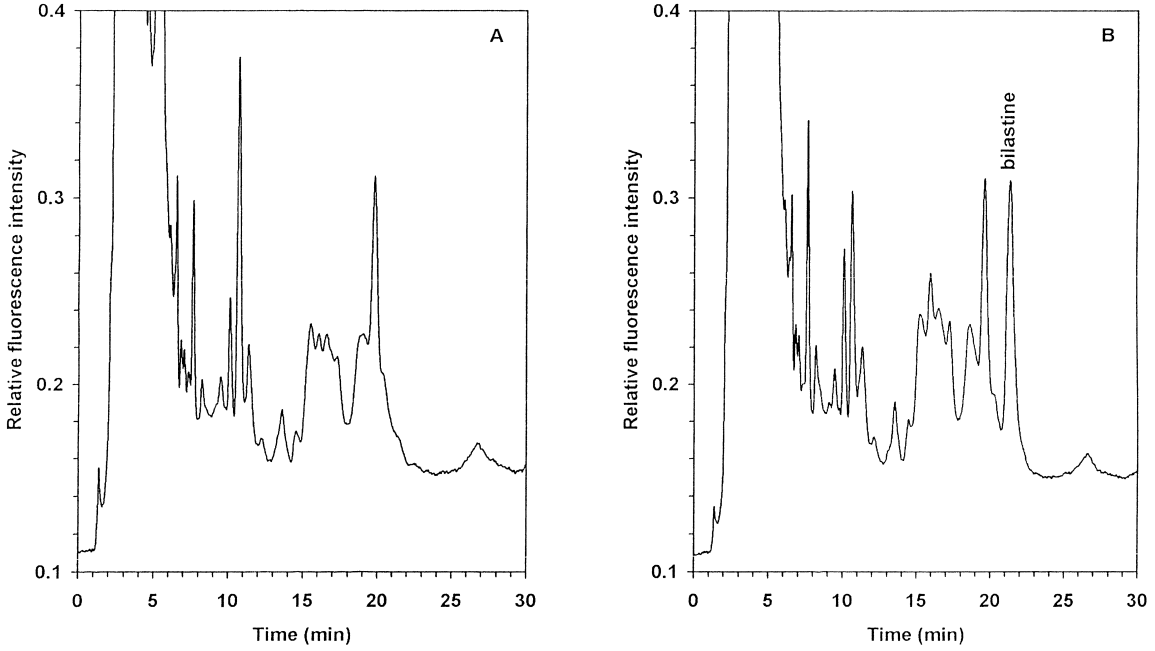

Fig. 2. Typical chromatograms obtained for: (a) blank rat faeces and (b) rat faeces spiked with 0.05 μg/g of bilastine.

Table 1
Recovery, within-day and between-day repeatability for the assay of bilastine in rat faeces

	Day 1	Day 2	Day 3	Between-day repeatability
0.05 μg/g				
Mean (μg/g)	0.0523	0.0496	0.0570	0.0534
SD (μg/g)	0.0024	–	–	0.0037
RSD (%)	4.6	–	–	7.0
Mean recovery (%)	104.6	99.1	114.0	106.8
n	3	1	1	3
0.5 μg/g				
Mean (μg/g)	0.538	0.467	0.473	0.494
SD (μg/g)	0.010	–	–	0.042
RSD (%)	1.9	–	–	8.4
Mean recovery (%)	107.5	93.3	94.7	98.8
n	3	1	1	3
5 μg/g				
Mean (μg/g)	4.568	5.129	4.374	4.72
SD (μg/g)	0.072	–	–	0.38
RSD (%)	1.6	–	–	8.1
Mean recovery (%)	91.4	102.6	87.5	94.3
n	3	1	1	3

during 1 day at room temperature). No significant differences were observed showing the stability of bilastine throughout the entire analytical process.

Linearity of calibration was assayed using standard solutions of bilastine and spiked rat faeces at eight concentration levels between 0.05 and 10 μg/g. Good linear relations were attained in both cases within this range:

Standard solutions: Area =

$(-1.2 \pm 1.2) + (113.66 \pm 0.29) \cdot \text{Conc.}\ (\mu g/g)$

$(r^2 = 0.99996)$

Spiked rat faeces: Area =

$(-3.1 \pm 4.1) + (108.3 \pm 1.0) \cdot \text{Conc.}\ (\mu g/g)$

$(r^2 = 0.9995)$

The absolute recovery calculated from the difference between these two calibration lines was 95.2%. The limit of detection (LOD), calculated as the bilastine concentration in faeces that produced a chromatographic peak with a height three times the baseline noise ($3S/N$), was 0.01 μg/g.

The precision of the bioanalytical method, expressed by the RSD, was estimated by measuring the within-day and between-day repeatabilities of the analysis for blank rat faeces samples spiked at three concentration levels (0.05, 0.5 and 5 μg/g) with three replicates. As shown in Table 1, the within-day repeatabilities were less than 5% and the between-day repeatabilities less than 9%.

The method was successfully applied to the excretion studies of bilastine in rat. A large number of samples were analysed, obtaining well defined values for concentration levels as low as 0.03 μg/g, value at which we can place the limit of quantification of the proposed method. Example chromatograms are shown in Fig. 3, corresponding to faeces samples collected during the period 0–8 h after a single oral administration of 20 mg/kg of bilastine and 0–8 h after a single intravenous administration of 10 mg/kg of bilastine. The obtained concentrations in these samples were 0.46 and 0.13 μg/g, respectively.

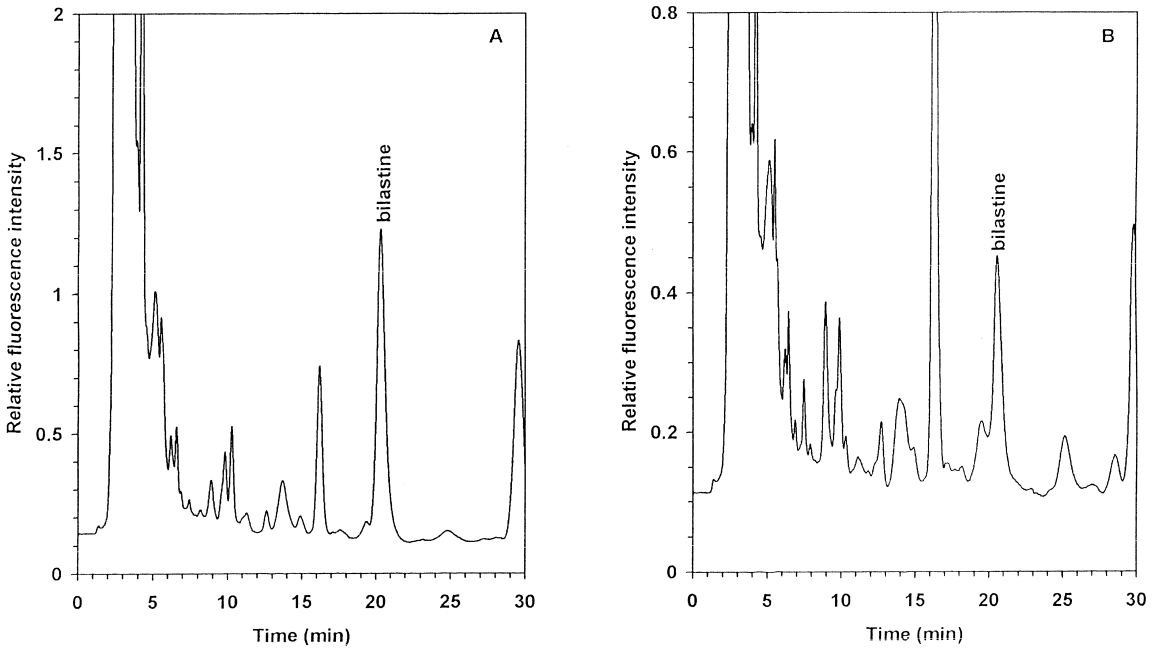

Fig. 3. Chromatograms corresponding to: (a) a faeces sample collected during the period 0–8 h after a single oral dose of 20 mg/kg, and (b) a faeces sample collected during the period 0–8 h after a single intravenous administration of 10 mg/kg.

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