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Gas–liquid chromatography of hydroxamic acids in biological samples

Detection and quantitation of Bufexamac* and one of its major metabolites in plasma and urine

Bufexamac (*p*-butoxyphenylacethydroxamic acid), a potent anti-inflammatory drug[1], is metabolically converted into *p*-butoxyphenylacetic acid (BPAA) at a rate which is mainly dependent on the animal species studied and the route of administration chosen[2–4]. In man, both Bufexamac and BPAA have been detected in blood and urine after both oral and rectal administration of the native drug[3,5]. This paper describes a gas–liquid chromatographic (GLC) method for the assessment, in man, of both compounds in plasma and in urine; calibration curves are discussed. For plasma, a procedure for the simultaneous determination of the two above mentioned compounds in a single small sample has been developed.

Methods

Bufexamac has been found as a free compound in blood as well as in urine, while BPAA is present as a conjugate[4]. Therefore hydrolysis is required to free the BPAA prior to assay. On the other hand, using the ^{14}C-labelled compounds, it has been shown that Bufexamac can be selectively extracted (90–100%) at pH 8 with methylisobutylketone (MIBK) while free BPAA can be extracted at an acidic pH with the same solvent (about 100%). Since HCl-hydrolysis converts Bufexamac into BPAA, the former must thus be extracted first in the case of simultaneous determination.

Plasma (simultaneous assay). 4 ml of plasma is taken containing known amounts of Bufexamac and BPAA, and 4 ml of 66.6 m*M* Sörensen phosphate buffer at pH 8 are added. The 8 ml sample thus obtained is extracted twice with MIBK (1:2) for 15 min, using an automatic shaker (Gerhardt). The separated aqueous phase is then hydrolyzed at 100° for 20 min by adding 0.7 ml of 12 *N* HCl and, thereafter, is again extracted twice with MIBK (1:2). The two solvent phases, dried with anhydrous Na_2SO_4, are separately evaporated under reduced pressure (Rotavapor, Büchi) at 35°. The residues are dissolved in 20 μl of 1,4-dioxane containing 10 μg of marker (internal standard). For Bufexamac the marker is 3-chloro-4-propyloxyphenylacethydroxamic acid (CPPA) while for BPAA, 3-chloro-4-allyloxyphenylacetic acid (Alclofenac**) is used. Silylation of both types of sample is performed by adding 40 μl of N,O-bis-(trimethylsilyl)-acetamide (BSA, Pierce Chemical Co., No. 49011). 3 μl of each mixture are injected into the gas chromatograph after standing overnight at room temperature.

Urine (separate assay). As the specimen of urine is generally very large, Bufexamac and BPAA are assayed on separate aliquots. Bufexamac is determined in 10 ml urine containing known amounts of the drug, diluted with an equal volume of phosphate buffer at pH 8. The procedure is similar to that described for plasma except that the residue is dissolved in 50 μl of 1,4-dioxane containing 50 μg of CPPA (marker) and that 50 μl of BSA are added for the the formation of the derivative. 2 μl of the mixture are injected into the gas chromatograph.

BPAA is assayed in 10 ml of undiluted urine hydrolyzed with 0.5 ml of 12 *N* HCl

* Droxaryl®, Continental Pharma s.a., Brussels, Belgium.
** Mervan®, Continental Pharma s.a., Brussels, Belgium.

(20 min at 100°). After extraction with MIBK, the residue is dissolved in 250 μl of 1,4-dioxane containing 625 μg of Alclofenac (marker). Silylation is carried out by adding 250 μl of hexamethyldisilazane (HMDS, Pierce Chemical Co., No. 85770) and 125 μl of trimethylchlorosilane (TMCS, Pierce Chemical Co., No. 88530). 1 μl of the mixture is injected into the gas chromatograph.

A Packard (series 7400) gas chromatograph is used. The unit is equipped with a dual flame ionisation detector operating at 250°. Glass columns, 2 m long and 4 mm I.D., packed with Supelcoport 80–100 mesh (Supelco, Inc.) coated with S.E. 30 (3 %), are used. The carrier gas is nitrogen (60 ml/min). The chromatographic separation is performed at 130° for Bufexamac and 140° for BPAA. The inlet temperature is 250°. Under these conditions retention times for Bufexamac, CPPA, BPAA and Alclofenac are respectively 15.6, 20.5, 17.5 and 22.6 min.

Results

Calibration curves for Bufexamac and BPAA in plasma (simultaneous determination) and in urine (separate assay) are shown in Figs. 1 and 2. Known amounts (see figures) of both compounds were dissolved in the plasma and urine before any manipulation. These amounts have been chosen in order to simulate the concentrations determined by liquid scintillation counting in man, treated with ^{14}C-labelled Bufexamac[5].

The differences in the procedures used (*i.e.*), dilutions, silylating agents, temperature, etc.) for the two compounds when different samples are handled, are dictated by the necessity of avoiding the presence of interfering peaks in the regions occupied by the four compounds tested.

For the two hydroxamic acids assayed, the Lossen rearrangement according to VAGELOS *et al.*[6] has also been investigated but this method was satisfactory for urine only. In plasma, cumbersome interfering peaks have been noticed.

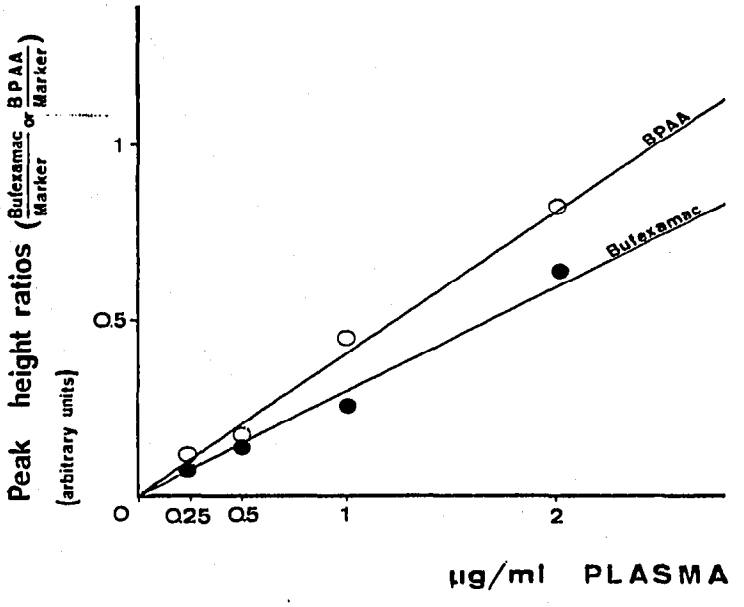

Fig. 1. Calibration curves for Bufexamac and BPAA in plasma (4 ml) of humans (simultaneous assay). GLC assessments are based on the Bufexamac/marker and BPAA/marker peak height ratios. Each point is the mean of three determinations: Bufexamac, ●; BPAA, ○.

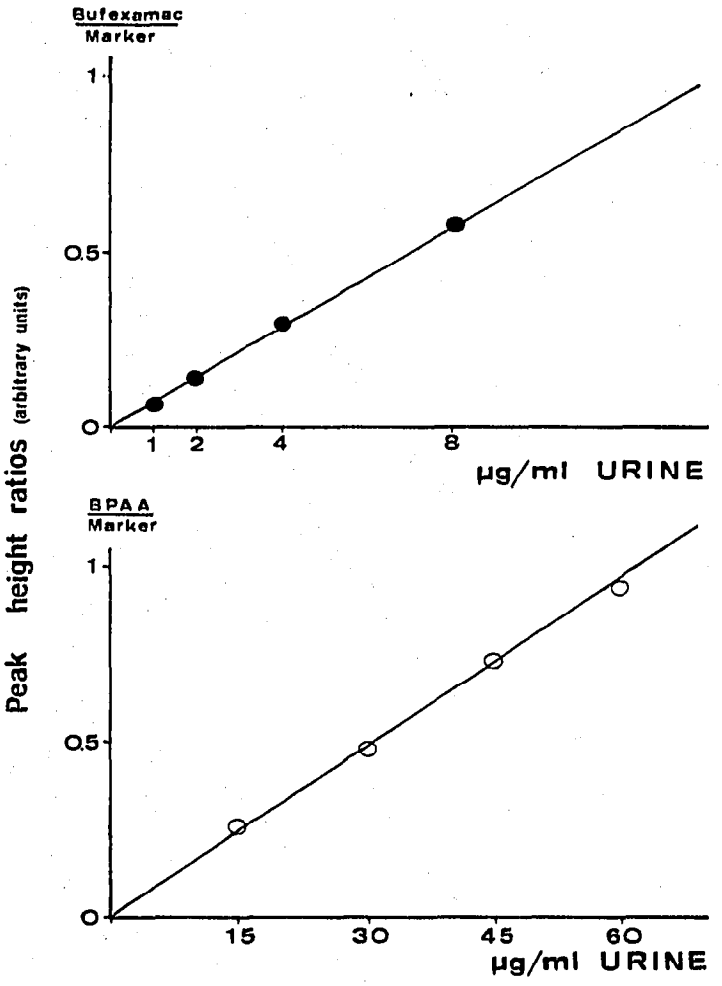

Fig. 2. Calibration curves for Bufexamac and BPAA in urine (10 ml) of humans (separate assays). GLC assessments are based on the Bufexamac/marker and BPAA/marker peak height ratios. Each point is the mean of three determinations: Bufexamac, ●; BPAA, ○.

The calibration curves presented in this note show that concentrations as low as 250 ng/ml can be assayed in plasma and urine with sufficient reliability. Results obtained with actual samples will be discussed elsewhere.

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