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Note

Determination of butanilicaine in horse plasma and urine by extractive benzoylation and gas chromatography with a nitrogen–phosphorus detector*

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Butanilicaine [(2-(butylamino)-N-(2-chloro-6-methylphenyl)-acetamide)] is an amide-type local anaesthetic for veterinary use, with a faster and longer activity than procaine[1].

A previously described method[2] for the analysis of butanilicaine in urine was based on colorimetry. Earlier studies concerning the metabolism of local anaesthetics[3–5], especially butanilicaine breakdown in the rat[2,6], reveal that this drug is expected to be extensively metabolized in the horse. Therefore the complicated colorimetric method should lack sensivity for the determination of butanilicaine in horse plasma and urine.

Although a gas chromatographic (GC) screening procedure with nitrogen specific detection enabling the detection of eleven local anaesthetics in horse urine and plasma was recently published[7], this method could not been used for the quantitative determination of butanilicaine owing to peak tailing.

In the present study an extractive benzoylation reaction based on the Schotten–Baumann procedure has been utilized for the quantitative determination of butanilicaine in biological fluids.

EXPERIMENTAL

Materials

Butanilicaine triphosphate and the internal standard 2-(butylamino)-N-(2-methyl-4-chlorophenyl)acetamide. HCl were supplied by Hoechst (Frankfurt, G.F.R.). Stock solutions were prepared in double-distilled water.

Pentafluorobenzoyl chloride (PFBCl) was obtained from Aldrich Europe; trifluoroacetic acid anhydride (TFAA) and pentafluoropropionic acid anhydride (PFPA) were purchased from Pierce. The triethanolamine–cyclohexane extraction solvent (CH-TEA) was prepared by briefly refluxing cyclohexane with small amounts of triethanolamine, cooling and separating the two phases. The ammonium buffer was a saturated NH_4Cl solution adjusted to pH 9.4 with NH_4OH.

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All glassware was silanized as described earlier[8], and the organic solvents (analytical grade) were freshly distilled before use. Dilutions were made with a Hamilton digital diluter/dispenser.

Gas chromatography

A Varian 3700 equipped with ^{63}Ni and nitrogen-specific detectors and connected to a Varian CDS 111 integrator was used. The glass column (150 × 0.25 cm I.D.) was packed with 3% OV-7 on Chromosorb W HP, 80–100 mesh. The oven temperature was set at 235°C. The injector and detector temperatures were kept at 250°C and 300°C, respectively. Nitrogen (25 ml/min) was used as carrier gas.

Mass spectrometry

A mass spectrum was obtained on a HP 5995 apparatus, the column being a 25-m SP-2100 fused-silica column.

Methods

For the determination of butanilicaine in horse plasma or urine, 2 ml of the biological fluid, 50 μl of the aqueous internal standard solution (10 μg/ml) and 0.2 ml of ammonium buffer were mixed with 6 ml CH-TEA and 10 μl of PFBCl (5% in cyclohexane) for 5 min. After a brief centrifugation, 5 ml of the organic phase were transferred to a tapered test-tube and evaporated to dryness under nitrogen at 40°C. The residue was redissolved in 50 μl of ethyl acetate, and 1 μl was injected into the gas chromatograph (nitrogen–phosphorus detection mode).

A standard graph was prepared by treating known amounts of butanilicaine triphosphate in plasma according to this procedure.

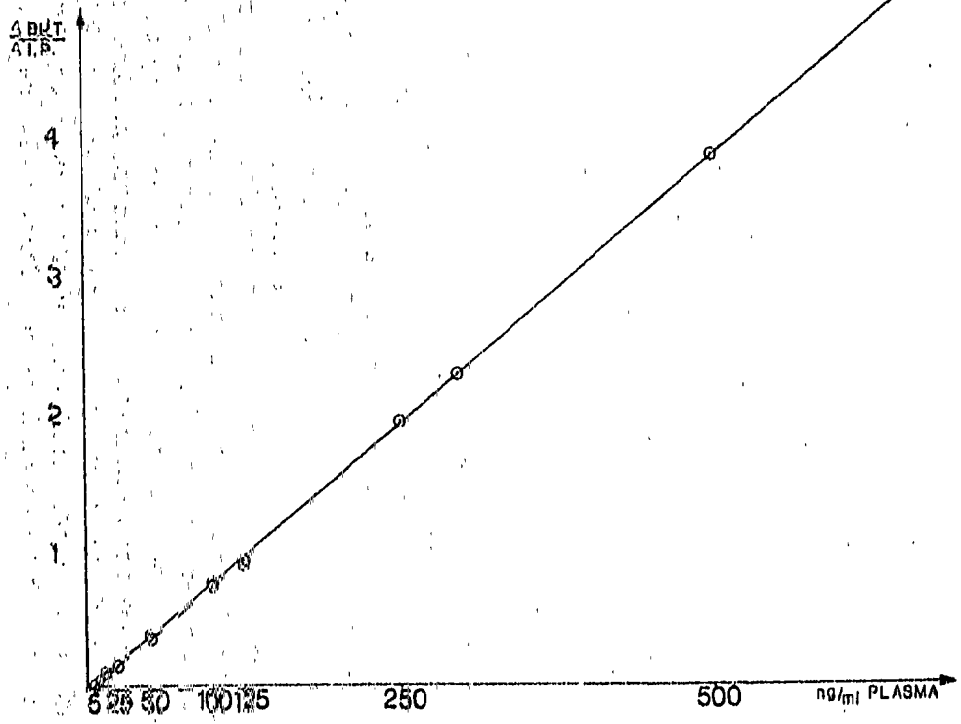
The recoveries were obtained by adding different amounts of butanilicaine triphosphate to 2 ml of plasma or urine and extractive benzoylation (rotating or vortexing) followed by the addition of the internal standard as a methanolic solution, evaporation and subsequent reaction with PFBCl.

RESULTS AND DISCUSSION

Because the gas–liquid chromatographic separation of compounds containing amino groups generally results in peak tailing due to spurious adsorption onto the column, several approaches have been used for the derivatization of the amine function in butanilicaine and the isomeric internal standard.

The acylation with TFAA and PFPA was not complete at room temperature and the compounds formed were easily hydrolysed. Therefore benzoylation of the secondary amino group in butanilicaine was tried.

Benzoyl derivatives can be made from alcohols, thiols and amines by treating the residue obtained by benzene extraction with pyridine and benzoyl chloride and shaking over several hours[9]. The Schotten–Baumann procedure, whereby an alkaline glycerol solution was shaken with benzoyl chloride, followed by extracting the derivatives, was applied by Decroix *et al.*[10]. The Schotten–Baumann type acylation reaction was also used for the determination of phenolic amines in water or plasma[11]. PFBCl derivatives are generally made with trimethylamine as catalyst[12] or at elevated temperatures[13,14]. In this work, however, a Schotten–Baumann reaction was performed



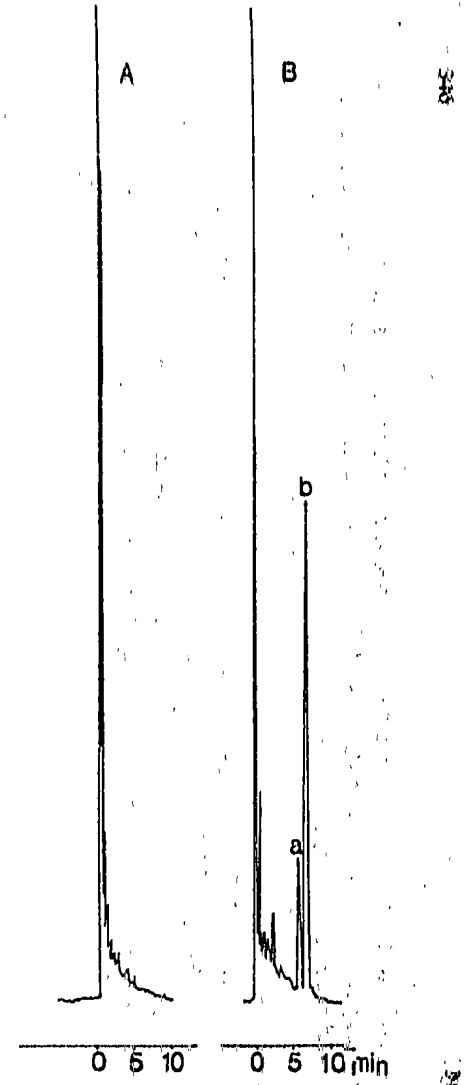


Fig. 1. Standard graph obtained by spiking horse plasma.

Fig. 2. Typical chromatograms of horse plasma. A, Blank control plasma; B, spiked plasma. Peaks a and b are the N-pentafluorobenzamides of butanilicaine and the internal standard, respectively.

using PFBCl with simultaneous extraction at room temperature of the pentafluorobenzamide formed. The basic catalyst was the water-soluble triethanolamine. The derivative has good GC properties, and the organic phase can be washed with 0.01 *N* NaOH without noticeable hydrolysis.

Although the pentafluorobenzoyl group appears to be the one that confers the greatest sensitivity for electron-capture detection of amines[15], this detection method could not be used here. Indeed, the formation of the strongly electron-capturing pentafluorobenzoic acid (PFBH) causes serious GC interference. Although PFBH can be removed by extracting the organic phase with 1 *N* NaOH[16], this procedure could not be used here owing to the hydrolysis of the benzamide and butanilicaine itself at this pH. Nevertheless, the use of the nitrogen-specific detector enables an acceptable detection limit to be achieved.

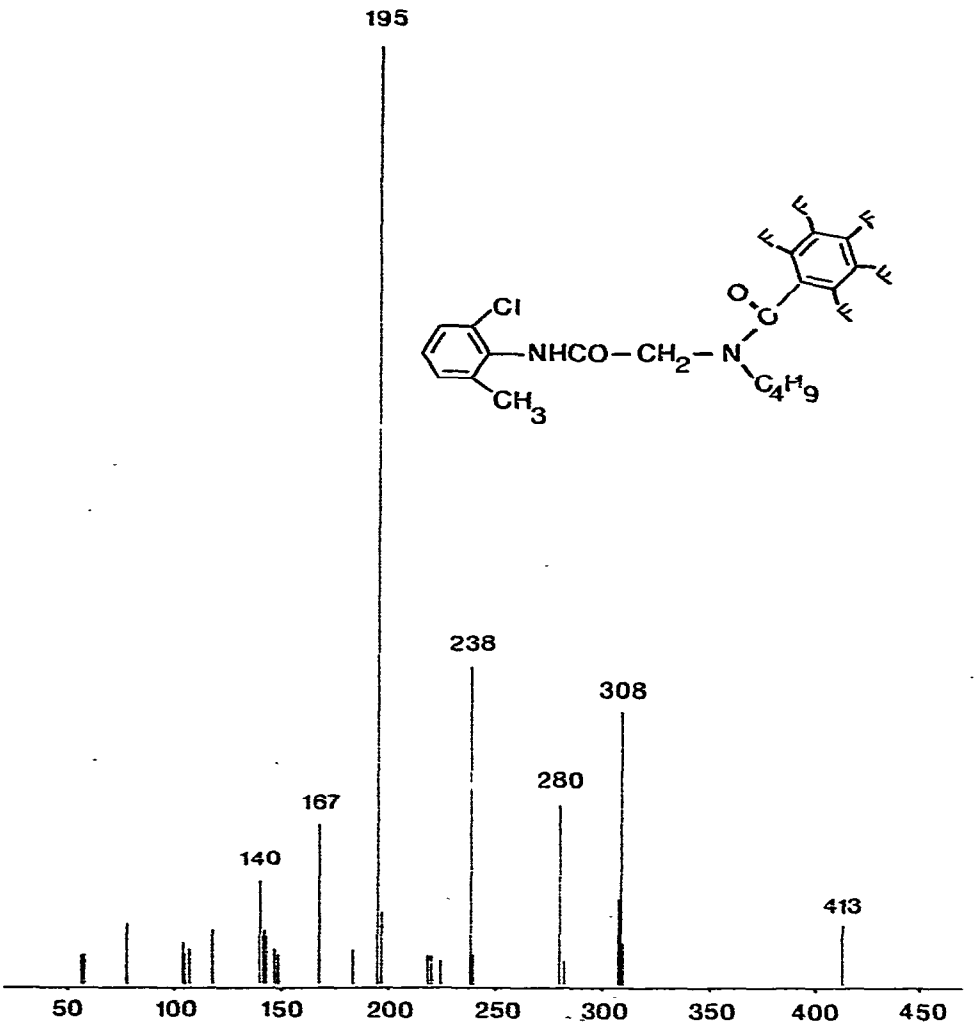


Fig. 3. Electron impact mass spectrum of the pentafluorobenzamide of butanilicaine.

TABLE I

RECOVERIES OF BUTANILICAINE ADDED TO HORSE PLASMA OR URINE

Butanilicaine added to 2 ml	*Recovery*		
	Plasma		*Urine*
	Shaking 5 min	*Vortexing, 1 min*	*Vortexing, 1 min*
250 ng	183 ± 12 ng	193 ± 23 mg	210 ± 10 ng
500 ng	452 ± 36 ng	396 ± 18 ng	389 ± 10 ng
1250 ng	1108 ± 48 ng	944 ± 27 ng	1017 ± 60 ng
Recovered, pipetting 5 ml out of 6 ml	85 ± 9% $n = 17$	77 ± 5% $n = 16$	81 ± 4% $n = 15$
Absolute recovery	±102%	±92%	±97%

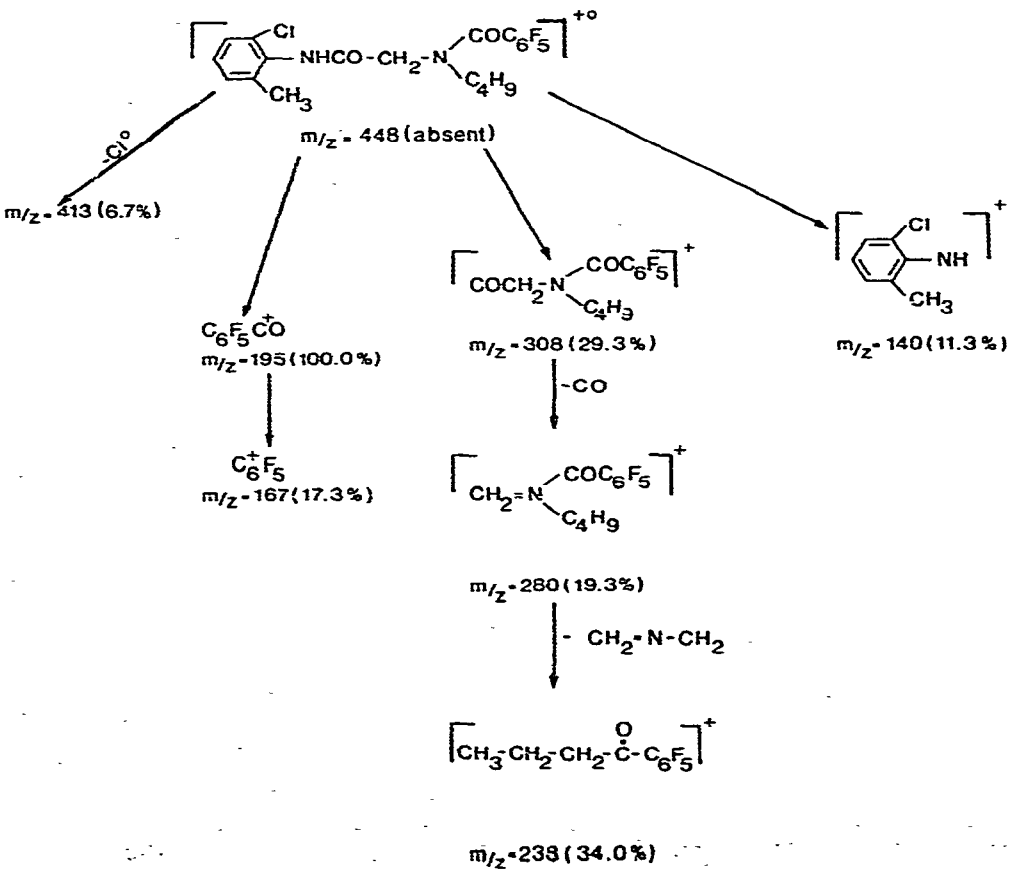


Fig. 4. Postulated mass fragmentation pattern of the N-pentafluorobenzamide of butanilicaine.

The extractive pentafluorobenzoylation of butanilicaine and the internal standard was complete after *ca.* 5 min at room temperature. The recoveries (Table I) were nearly quantitative.

A standard graph with a linear concentration range in the interval 5–500 ng/ml is presented in Fig. 1 ($r = 0.999$, $n = 38$). With 2 ml of plasma, the detection limit was evaluated at 5 ng of butanilicaine triphosphate. Fig. 2 shows a typical chromatogram obtained by processing blank and spiked horse plasma as described in the Experimental section.

The mass spectrum of the pentafluorobenzamide of butanilicaine did not give a molecular ion (Fig. 3). However, diagnostic ions at $m/z = 413$, 308, 280, 238 and 140 were observed. The postulated structures for these ions are shown in Fig. 4.

CONCLUSIONS

With triethanolamine as catalyst, the extractive benzoylation under Schotten–Baumann conditions of butanilicaine with PFBCl and subsequent GC with nitrogen-specific detection is a simple, rapid and sensitive technique for the determination of butanilicaine in plasma and urine.

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