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Note

A rapid sensitive determination of Carprofen and Zomepirac using thin-layer chromatography and gas chromatography–mass spectrometry

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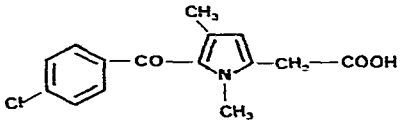
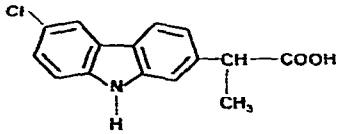
Zomepirac [5-(4-chlorobenzoyl)-1,4-dimethyl-H-pyrrole-2-acetic acid]



mol.wt. 291.74

and Carprofen [2-(6-chlorocarbazol-2-yl)propionic acid]



mol.wt. 273.72

are two new non-steroidal antiinflammatory analgesics. Both drugs are weak organic acids with a pK_a of 4.37 for Zomepirac and 4.7 for Carprofen, and a plasma–protein binding of 98%[1–3]. Less than 10% of the substances are eliminated in urine as the free drug, the rest being mainly glucuronide metabolites.

The objective of the present study was to develop a simple rapid, specific and sensitive thin-layer and gas chromatographic (GC) assay for the determination of Zomepirac and Carprofen in urine by routine screening for analytical doping control in horses and toxicological analyses.

MATERIAL AND METHODS

Materials

Glucuronidase/arylsulphatase was purchased from Boehringer (Mannheim, G.F.R.). All other chemicals were of highest purity available either from Fluka or

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Merck. Zomepirac was a gift of Cilag (Shaffhausen, Switzerland); Carprofen was provided by Hoffmann-La Roche (Basle, Switzerland). The MN-silica gel G for thin-layer chromatography (TLC) was from Macherey, Nagel & Co. (A. Radmacher, Düren, G.F.R.).

Hydrolysis of conjugated drugs

A 5-ml volume of urine was adjusted to pH 5.5 with 3 *M* acetic acid and then 0.5 ml of 10% (v/v) 2 *M* acetoacetic buffer (pH 5.5) were added. Glucuronidase/arylsulphatase (0.015 ml) was pipetted into the urine. The mixture was placed in a thermostatted water-bath at 40–45°C overnight.

Extraction and derivatization

To the 5-ml volume of hydrolysed urine (pH 4.0), 10 ml of diethyl ether were added and the mixture shaken mechanically for 10 min. The organic layer was then removed. The procedure was repeated. The diethyl ether was then evaporated to dryness at room temperature by a gentle stream of nitrogen. The residue was dissolved in 50 μl isopropanol. A 20-μl aliquot of this extract was methylated[4] and used for GC and for GC–mass spectrometry (MS).

Thin-layer chromatography

TLC was performed on 0.25-mm precoated layers of silica gel 60 and 60-F_{254}. 5-μl aliquots of urine extracts in isopropanol solution were spotted. After spotting the plates were developed in a chromatographic tank using the ascending technique. The run of the developing solvent, chloroform–methanol–water (70:30:2), was 10 cm. The developed layers were air-dried. All chromatograms were sprayed with a 1:2 mixture of 10% aqueous $FeCl_3$ and 1% aqueous K_3 $[Fe(CN)_6]$ and heated at 110°C for 5–10 min. The reagents have to be prepared immediately before use.

Gas chromatography–mass spectrometry

GC–MS analysis was carried out on a MAT 212 chemical ionization mass spectrometer interfaced to a Varian 3700 gas chromatograph. The glass capillary column (25 m × 0.35 mm I.D.) was wall coated with OV-17. The injection port temperature was 235°C, and the samples were injected at an oven temperature of 80°C. The temperature was increased at 10°C/min up to 250°C Helium was used as the GC carrier gas.

A SS MAT 188 interactive data system was used to control the mass spectrometer during selected ion monitoring and to calculate the heights of peaks in selected ion chromatograms.

RESULTS AND DISCUSSION

Thin-layer chromatography

After comparing various developing solvents we found that the mixture reported not only separated Zomepirac and Carprofen, but that these compounds could also be identified in the presence of other well known non-steroidal antiinflammatory drugs such as Diclofenac, Mefenamic acid and Ketoprofen (Table I).

After spraying the silica gel 60 TLC plates, a blue colour resulted for all

TABLE I

R_F VALUES AND CHARACTERISTIC COLOURS

Solvent: chloroform–methanol–water (70:30:2).

Compound	*On silica gel 60*		*Fluorescent plate*
	R_F	*Colour with 10% $FeCl_3$–1% $K_3[Fe(CN)_6]$ (1:2)*	
Carprofen	0.49	Blue	Dark spot
Zomepirac	0.32	Blue	Dark spot
Diclofenac	0.55	Blue	Dark spot
Mefenamic acid	0.70	Blue	Dark spot
Ketoprofen	0.10	–	Dark spot

drugs but not for Ketoprofen. A differentiation in colour for the various drugs was not attainable. TLC is however a simple and inexpensive procedure which gives preliminary information in the screening method within a very short time.

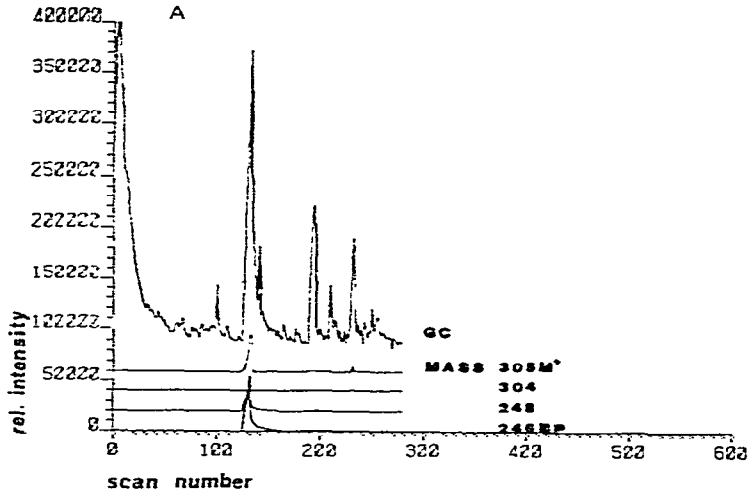
GC–MS identification of derivatives

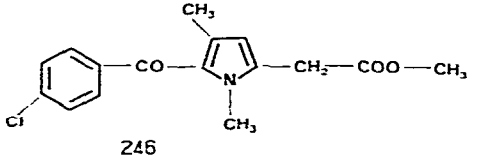
We injected the methylated urinary extract directly into the column and found one peak for Zomepirac which corresponded exactly with the standard drug solution (Fig. 1A, B and C). The corresponding retention time and the fragments of scanning number 145, *m/e* 246 (100%), 305 (40%) and 111 (20%), after derivatization in Fig. 1B and C, demonstrate the identity of Zomepirac.

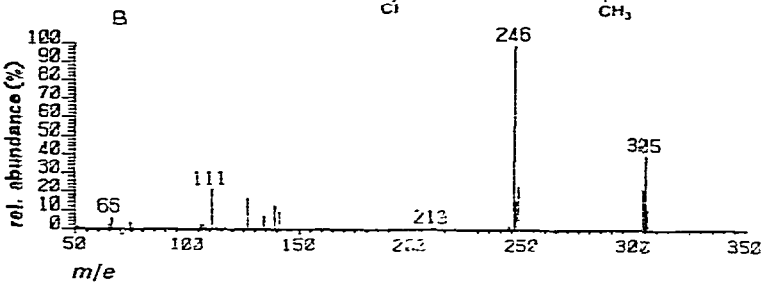
Evaluating urine samples with the ^{14}C-method. Wu *et al.*[5] showed that Zomepirac-glucuronide accounted for 56.8% of dose, whereas free Zomepirac represented 21.8%, hydroxyzomepirac 5.5%, and 4-chlorobenzoic acid accounted for the remainder of the identified metabolites. By our extraction method performed after hydrolysis we could detect only free Zomepirac but no other metabolites.

Carprofen is excreted in urine as Carprofen-glucuronide (75%), the free drug appearing in very small quantities (3%)[6]. The methylated urine extract showed one main peak in the chromatogram (Fig. 2A). The analysis of scanning number 245 in comparison with the methylated standard substance Carprofen proved that the drug was excreted without any chemical degradation. Metabolites of Carprofen could not be detected in the acid fraction. In Fig. 2B and C the identitity of methylated Carprofen is demonstrated for the standard drug and for a urinary extract from patients who had taken 300 mg of the drug p.o. daily for 10 days.

In conclusion, we could detect both drugs without interference of physiological substances in the GC–MS system. The same results were found with the GC system alone using an OV-101 glass column and a flame ionization detector. Thus, the detection of Zomepirac and Carprofen is possible without any difficulties. The method is sensitive. In urine extractions without hydrolysis Carprofen as well as Zomepirac were found in small quantities. The detection in serum is also possible if pharmacokinetic factors are respected. In contrast to several analytical procedures for the detection of non-steroidal analgesics described by other authors[7–9], the present method seems extremely suitable for quick screening of urine and blood samples.







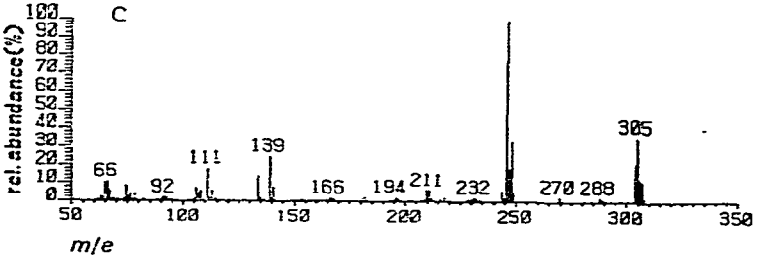
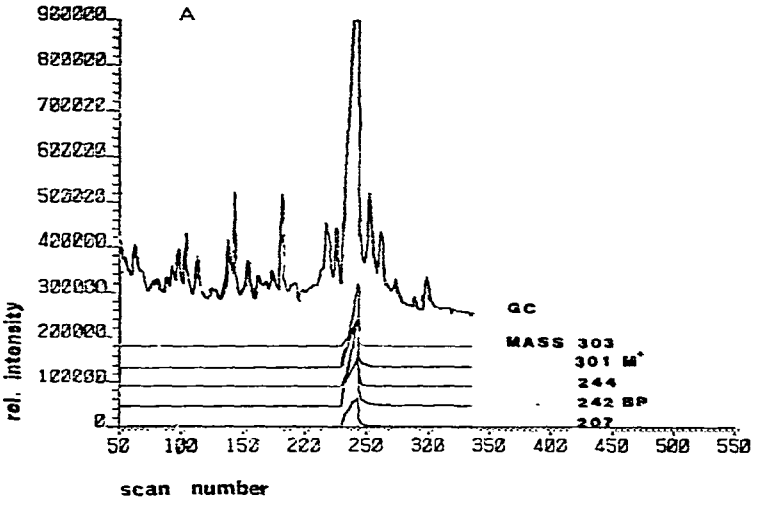
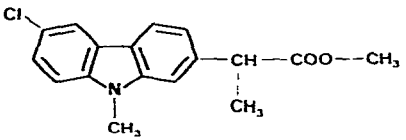
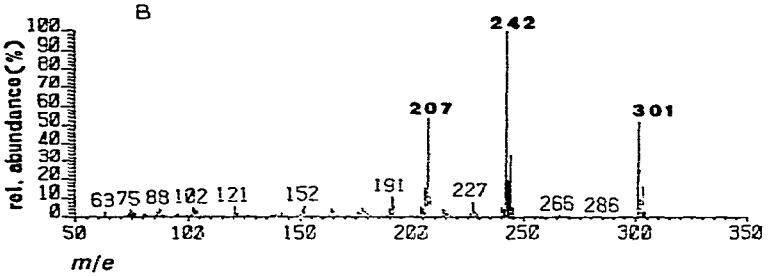


Fig. 1. GC–MS or urinary extract containing Zomepirac after methylation (A) and mass spectra of Zomepirac standard (B) and urinary extract from patients having taken 100 mg Zomepirac p.o. daily during 3 weeks (C).







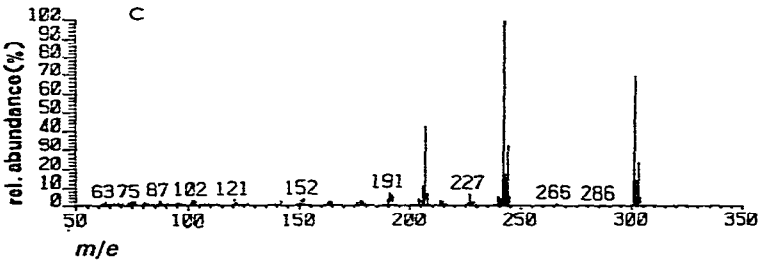


Fig. 2. GC–MS of urinary extract containing Carprofen after methylation (A) and mass spectra of the standard drug Carprofen (B) and of a urinary extract (C) from patients (having taken 300 mg Carprofen p.o. daily during 10 days).

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