Journal of Chromatography, 278 (1983) 225—227
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1838

Note

Determination of a novel antitussive agent 2′,4′-dimethyl-6′-methoxy-3-(2-methylpiperidyl)-propionaldehyde in plasma by high-performance liquid chromatography

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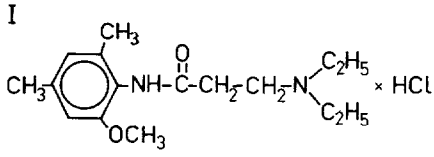
(First received May 3rd, 1983; revised manuscript received June 27th, 1983)

Opium alkaloids are the most effective antitussive compounds available. They have, however, several well-established side-effects and a risk of abuse as well [1]. Therefore a need for a new drug is evident.

A novel compound, 2′,4′-dimethyl-6′-methoxy-3-(2-methylpiperidyl)-propionaldehyde (OR K-242 hydrochloride) (Fig. 1, II), which is chemically related to lidocaine, was found in several types of animal experiments to be an effective inhibitor of the cough [2]. To establish the drug levels in plasma for the use in experimental and clinical pharmacokinetics a simple high-performance liquid chromatographic (HPLC) assay was developed.

I



II

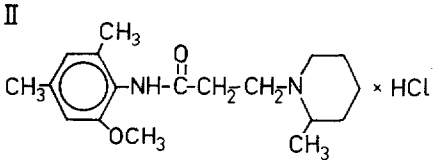


Fig. 1. Structures of the internal standard (I) and OR K-242 (II).

EXPERIMENTAL

Reagents

OR K-242 hydrochloride and the internal standard OR K-269 hydrochloride were obtained from Orion Pharmaceutical (synthesized by Ms. A. Pippuri

0378-4347/83/$03.00

and Dr. E. Honkanen, Research Center, Espoo, Finland). Chemical structures are shown in Fig. 1. Acetonitrile, HPLC grade S, was purchased from Rathburn Chemicals (Walkerburn, U.K.) and dichloromethane, Uvasol grade from E. Merck (Darmstadt, F.R.G.). All inorganic reagents were of analytical grade and purchased from commercial sources.

Sample preparation

Plasma samples (0.2—0.5 ml) were spiked with 125 ng of the internal standard. Plasma was alkalinized with 0.1 ml of 1.0 *M* sodium hydroxide in stoppered tubes and extracted with 6 ml of dichloromethane by shaking 10 min with a universal laboratory shaker. After centrifugation (5 min at 1100 *g*) 5 ml of the organic layer were evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 0.1 ml of the mobile phase and 20 μl were injected into the HPLC column.

Chromatography

The modular liquid chromatographic system consisted of a Waters Model 6000 A pump, a Waters Intelligent Sample Processor (WISP) Model 710 B (Waters Assoc., Millford, MA, U.S.A.), a 250 × 4.5 mm 5-μm Spherisorb-nitrile column (Phase Separations, Queensferry, U.K.). The eluted components were detected by ultraviolet (UV) absorption at 214 nm with a Kratos Model 773 variable-wavelength detector (Kratos Analytical Instruments, Ramsey, NJ, U.S.A.). The elution was carried out isocratically at ambient temperature using 30% acetonitrile in 15 m*M* sodium phosphate, pH 4.0, at a flow-rate of 1.5 ml/min.

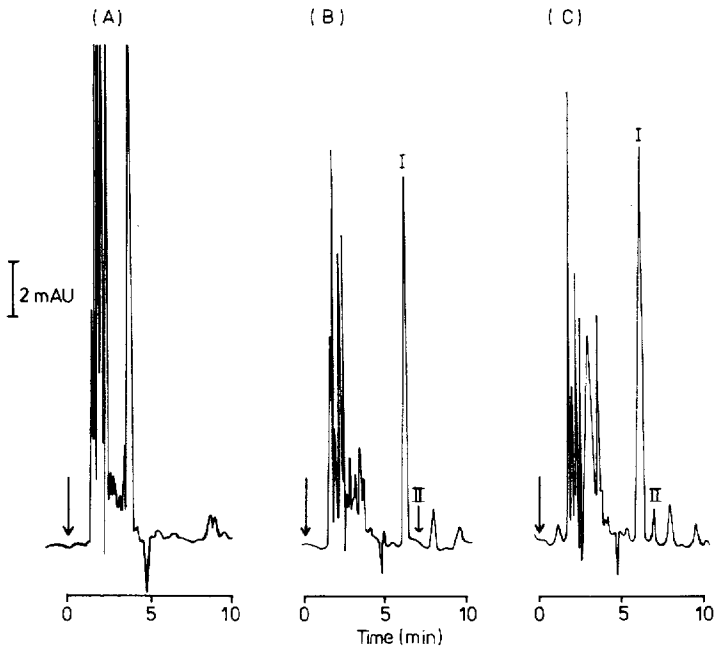


Fig. 2. Representative chromatograms of OR K-242 in plasma. (A) Blank plasma, (B) plasma spiked with 125 ng of internal standard, and (C) plasma containing 15 ng/ml OR K-242 and 125 ng/ml internal standard. Peaks: I = internal standard, II = OR K-242.

RESULTS AND DISCUSSION

Fig. 2 shows representative chromatograms of rabbit blank plasma (Fig. 2A), plasma spiked with 125 ng of the internal standard (Fig. 2B), and plasma 2 h after an intravenous dose of 20 mg/kg OR K-242 (Fig. 2C). The described extraction procedure and the low detection wavelength produced a couple of extra peaks in the chromatogram. These peaks were found to be due to impurities in the dichloromethane. Since they did not interfere with the compounds of interest, no further purification of the extraction medium was carried out. There was no interference with the common antitussive agent codeine, which eluted at 5.6 min (retention times for internal standard and OR K-242 were 6.2 and 7.0 min, respectively).

A plot of peak height ratio versus concentration of OR K-242 is linear over the range 10—1000 ng/ml and is described by the equation $y = 0.005x + 0.015$ ($r = 0.9997$). Detection limit with a signal-to-noise ratio of 3:1 is 2 ng/ml. The recovery of added OR K-242 (12.5 mg/ml and 1000 ng/ml, $n = 6$) from rabbit plasma was 95.5 ± 10.4% and 80.7 ± 4.4%, respectively. The precision of the assay was established by multiple measurements ($n = 10$) of quality control samples (100 ng/ml). The intra-assay mean ± S.D. was 95.5 ± 5.2 ng/ml, C.V. = 5.5%, and the inter-assay mean ± S.D. was 92.5 ± 6.8 ng/ml with C.V. = 7.3%.

The described HPLC assay with UV detection at 214 nm provides a fast, simple and sensitive method to determine plasma levels of the novel antitussive agent OR K-242. It has been successfully used to determine OR K-242 concentrations in plasma of different animal species necessary for experimental pharmacokinetic analysis.

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