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Screening for coumatetralyl in soft drinks by solid-matrix extraction and high-performance liquid chromatography with diode-array detection

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ABSTRACT

A method was developed for the rapid determination coumatetralyl in cola- and orange-type soft drinks, which includes extraction using solid-matrix column, clean-up by silica cartridge chromatography and reversed-phase high-performance liquid chromatography with diode-array detection. The recovery of coumatetralyl from 50 ml of soft drinks was better than 80% at spiking levels down to 50 μg/kg (ppb).

INTRODUCTION

Coumatetralyl [CAS Registry No. 5836-29-3; 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthalenyl)-2*H*-1-benzopyran-2-one] is an anticoagulant rodenticide with a subchronic LD_{50} (5 days) for rats of 0.3 mg/kg daily [1]. It is used as the active ingredient in numerous commercial rodent baits and concentrates. Recently, we investigated the presence of coumatetralyl in soft drinks in a case of suspected intentional poisoning of beverages from industrial production. A method for rapidly screening for this compound in a large number of samples of orange- and cola-type soft drinks was needed. About 400 samples from different regions of Italy had to be checked by five laboratories in about 2 weeks. The technique of choice for the determination of coumatetralyl is high-performance liquid chromatography (HPLC) [2,3]. However, to our knowledge, no method was available for the extraction and clean-up of coumatetralyl in soft drinks.

Although, the incident with which we had to deal seems unlikely to reoccur, we believe it is useful to report the development of a method for the rapid determination of coumatetralyl in cola-type soft drinks which includes extraction/clean-up on a diatomaceous earth cartridge and determination by reversed-phase HPLC. For orange-type soft drinks a further clean-up by silica gel cartridge chromatography was necessary before HPLC determination.

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EXPERIMENTAL

Reagents

Dichloromethane (analytical-grade reagent), redistilled from an all-glass apparatus, methanol and water (HPLC grade) were used.

Ready-to-use, disposable Chem Elut 2050 cartridges, filled with high-surface-area diatomaceous earth, were obtained from Analytichem International (Harbor City, CA, U.S.A.) and Millipore Sep-Pak silica cartridges from Water Assoc. (Milford, MA, U.S.A.).

Thin-layer chromatographic (TLC) plates, silica gel 60 F_{254}, 5 × 10 cm, with a 0.25-mm layer thickness (E. Merck, Darmstadt, Germany), were used as received.

Apparatus

The HPLC system was composed of a Perkin-Elmer Series 410 pump, a Rheodyne Model 7125 20-μl loop injector and a Perkin-Elmer Model 235 diode-array detector. Spectra were acquired in the range 195–370 nm. The monitoring wavelength in the chromatograms was 285 nm with 0.01 a.u.f.s.

A stainless-steel column (25 cm × 4.6 mm I.D.) packed with Spherisorb S5 ODS-2 (Phase Separations, Queensferry, U.K.) was used, and also a precolumn (4 cm × 2 mm I.D.) dry-packed with Perisorb RP-18, particle size 30–40 μm (E. Merck). The eluent was 0.1% acetic acid–methanol containing 0.1% acetic acid (40:60) at a flow-rate of 1 ml/min.

A rotatory evaporator (bath temperature 40°C; reduced pressure) was used.

Extraction

Transfer 50 ml of cola- or orange-type soft drink into a Chem Elut 2050 cartridge and wait 10 min to allow the liquid to absorb evenly. Add 50 ml of dichloromethane, wait for 3 min, then add two 50-ml portions of dichloromethane. Collect all the dichloromethane eluate and concentrate carefully to dryness. For cola-type soft drinks dissolve the residue in 1 ml of methanol and analyse by HPLC; for orange-type soft drinks, dissolve the residue in 1 ml of dichloromethane and continue with a silica gel cartridge clean-up (see below).

Silica gel cartridge clean-up

Fix a Sep-Pak silica gel cartridge to the outlet of a glass tube (15 cm × 15 mm I.D., terminated with a Luer tip), used as a solvent reservoir. Condition the cartridge with two 2-ml portions of dichloromethane. Transfer the sample solution into the cartridge reservoir and allow it to drain. Apply gentle pressure if the solution does not drain. Carry out two 1-ml washings of the sample vessel with dichloromethane and discard the eluates. Elute coumatetralyl with three 2-ml portions of dichloromethane, collect all the eluates and concentrate carefully to dryness. Dissolve the residue in 1 ml of methanol and analyse by HPLC.

HPLC analysis

Inject 20 μl of the sample into the HPLC system. Using the diode-array detector, compare the retention time and UV spectrum of the standard coumatetralyl with any peak appearing at the same retention time in the chromatogram of the sample.

RESULTS AND DISCUSSION

In the development of the method, the classical separating funnel extraction with dichloromethane was first tried, but was rapidly abandoned because of the formation of intractable emulsions.

In contrast, the solid-matrix extraction of soft drinks with dichloromethane proved to provide a rapid extraction with no emulsion formation; no reusable glassware was needed and the volume of solvent and amount of labour were significantly reduced compared with the separating funnel technique.

The chromatograms of samples of cola-type soft drinks contained some small peaks, but none interfered with the determination of coumatetralyl. Recovery experiments were carried out by spiking "blank" cola samples with standard coumatetralyl. Only one brand of cola was used for the recovery experiments. Samples serving as "blanks" were bought in regions where the batches of suspected poisoned cola had not been sold. These "blank" samples were checked and found to be free from peaks at the retention time of coumatetralyl. The mean recovery ± S.D. (n = 6) was 96 ± 6.5% and 81 ± 8.9% at 200 and 50 μg/kg, respectively. The limit of determination was calculated to be 10 μg/kg.

When orange-type soft drinks were analysed using the same method, they gave complex chromatograms with severe interferences at the retention time of coumatetralyl. It was therefore necessary to clean up these samples further. To this end the chromatographic behaviour of coumatetralyl was studied on non-activated silica gel plates, where coumatetralyl showed approximate R_F values of 0.42, 0.52, and 0.84

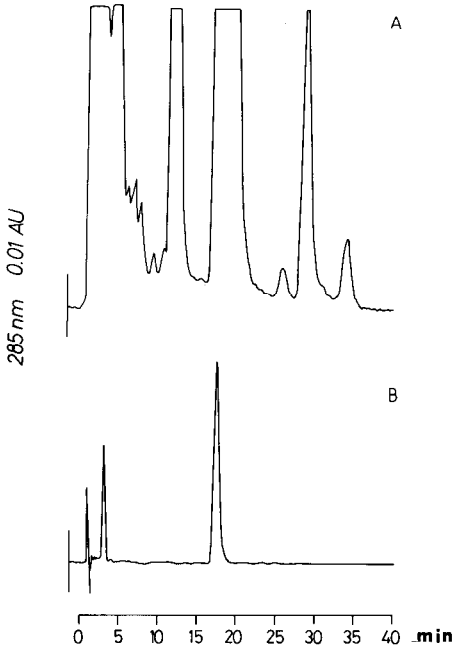

Fig. 1. HPLC of an orange-type soft drink (50 ml) spiked with 200 ppb of coumatetralyl. (A) Crude extract; (B) the same after TLC (wavelength 285 nm; 0.01 a.u.f.s.).

with dichloromethane, dichloromethane + 5% acetone and dichloromethane + 5% methanol, respectively. Therefore dichloromethane was used as the eluent for the separation of coumatetralyl from the raw orange extract obtained by solid-matrix extraction.

Fig. 1 shows the HPLC trace of the raw extract of an orange-type soft drink spiked with 200 ppb (μg/kg) superimposed on the trace obtained by scraping the band eluting on the silica gel TLC plate at the same R_F as the standard coumatetralyl. Thus, the TLC separation was able to remove the interferences and allowed the determination of coumatetralyl. A similar separation was obtained with a Sep-Pak silica gel cartridge, as can be seen in Fig. 2. For orange-type softdrinks spiked with coumatetralyl at 200 and 500 μg/kg levels, the mean recoveries $\pm$ S.D. (n = 6) were 92 $\pm$ 8.1% and 80 $\pm$ 9.2%, respectively, which are of the same order as those obtained with the shorter procedure for cola-type drinks.

The limit of detection (10 μg/kg) is considered to be acceptable for the particular problem concerned, because at this level the daily intake of a 60-kg person drinking 2 l of cola per day would be 0.0003 mg/kg body weight, which is one thousandth of the subchronic LD_{50} (5 days) for rats.

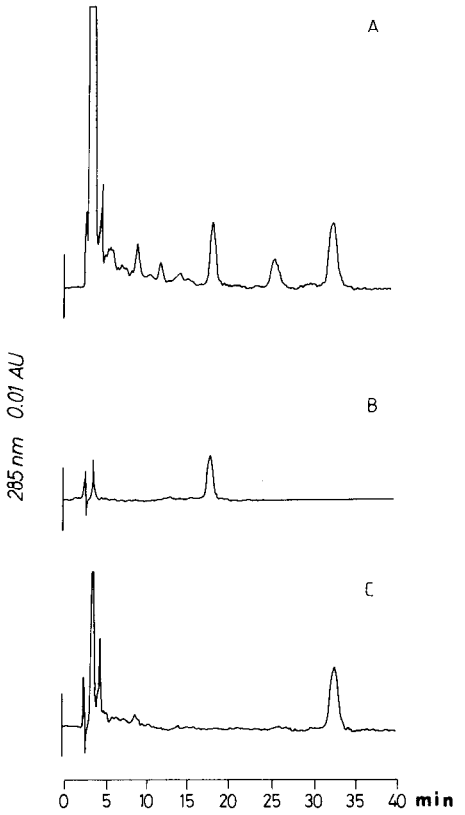

Fig. 2. HPLC of (A) an orange-type soft drink (50 ml) spiked with 50 ppb of coumatetralyl extract after silica gel cartridge clean-up; (B) 15 μg/ml coumatetralyl; (C) reagent blank in the described procedure (wavelength 285 nm; 0.01 a.u.f.s.).

CONCLUSIONS

The method developed offers several interesting features. It makes use of solid-matrix cartridges for extraction and clean-up, which reduces the amount of glassware and solvents needed. The extraction/clean-up procedure requires about 1 h for cola- and 1.5 h for orange-type soft drinks. The use of solid cartridges allows the parallel preparation of several sample extracts. Each HPLC run requires *ca.* 40 min. Hence this method is particularly suited for the screening of large numbers of samples.

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