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Short Communication

High-performance liquid chromatographic determination of imidocarb in cattle kidney with cation-exchange clean-up

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method for the determination of the antiprotozoal agent imidocarb in cattle kidney is developed. The drug is extracted from tissue with acetone in the presence of base. The extract is partitioned between saturated salt and chloroform and the organic layer evaporated to dryness. Clean-up is by cation-exchange solid-phase extraction on a carboxylic acid column. HPLC analysis is carried out on a Spherisorb S3W-PC18 column with ultraviolet detection at 260 nm. Average recoveries at the 0.05 and 0.01 mg kg^{-1} levels are 77.5 and 76.3%, respectively. The limit of detection is 0.001 mg kg^{-1}.

INTRODUCTION

Imidocarb (Fig. 1) is a dihydroimidazole-substituted carbanilide antiprotozoal drug. It is administered to cattle as the dipropionate salt at approximately 3 mg kg^{-1} body weight for the treatment of red water disease [1,2]. Although its potential toxicity is still under investigation, additional residue data are necessary in order to assess any risk that edible tissue residues of this compound present to the consumer. As far as known no procedures have been published for the determination of imidocarb in animal tissues. This paper describes a novel procedure for the determination of imidocarb in cattle kidney using weak cation-exchange solid-phase extraction (SPE) for clean-up and high-performance liquid chromatography (HPLC) as the determinative procedure.

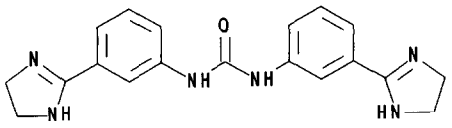

Fig. 1. Structure of imidocarb.

EXPERIMENTAL

Chemicals

Analytical-grade acetone, 1 *M* hydrochloric acid, sodium acetate trihydrate, anhydrous sodium carbonate, sodium chloride, 40% aqueous sodium hydroxide and anhydrous sodium sulphate were obtained from BDH (Poole, UK). HPLC-grade acetonitrile, dichloromethane and methanol and glass-redistilled-grade chloroform

were obtained from Rathburn (Walkerburn, UK). Dichlorodimethylsilane, sodium trifluoroacetate, tetramethylammonium chloride and trifluoroacetic acid were obtained from Aldrich (Gillingham, UK). Bond-Elut carboxylic acid (CBA) (500 mg/2.8 ml) cartridges (Analytichem International) were obtained from Jones Chromatography (Hengoed, UK) and were treated with 5 ml of methanol–(0.01 *M* sodium trifluoroacetate–0.01 *M* sodium acetate pH 7) (80:20, v/v) immediately prior to use. Imidocarb dipropionate reference standard was a gift from the Wellcome Foundation (Dartford, UK). The stock standard solution (1 mg/ml) was prepared by accurately weighing *ca.* 10 mg and dissolving it in 10 ml of methanol. Intermediate spiking solution (10.0 μg/ml) was prepared by diluting 100 μl stock standard solution to 10 ml with acetone. Working spiking solutions (1.0 and 0.2 μg/ml) were prepared by diluting 1 and 0.2 ml, respectively, of intermediate spiking solutions to 10 ml with acetone. Intermediate standard solution (10.0 μg/ml) was prepared by diluting 100 μl stock standard solution to 10 ml with HPLC mobile phase 1. Working standard solutions (1.0 and 0.2 μg/ml) were prepared by diluting 1 and 0.2 ml, respectively, of intermediate standard solutions to 10 ml with HPLC mobile phase 1.

Apparatus

An Ultra-Turrax homogeniser (Janke & Kunkel), L&R 140S ultrasonic bath, rotary evaporator (Büchi) with waterbath at 50°C, vortex mixer (Fisons), phase separation paper (Whatman PS-1) and MSI Cameo I 0.45-μm disposable syringe filters (Jones Chromatography) were used.

Glassware pretreatment

All non-disposable glassware was soaked in chromic acid prior to commencement of the analysis. Round-bottomed and pear-shaped flasks were washed with 10% dichlorodimethylsilane in dichloromethane, rinsed with methanol and dried immediately prior to use.

High-performance liquid chromatography

The HPLC system consisted of a Hewlett-Packard 1090 liquid chromatograph with 1040 diode-array detector controlled by a 79994A ChemStation. Samples (50 μl) were injected onto a Spherisorb S3W-C18 3-μm (Phase Separations) column (100 mm × 4.6 mm I.D.) and run with a mobile phase switch. Mobile phase 1 was (0.01 *M* sodium acetate–0.01 *M* sodium trifluoroacetate pH 7)–acetonitrile (85:15, v/v). Mobile phase 2 was (0.01 *M* sodium acetate–0.01 *M* trifluoroacetic acid–0.01 *M* tetramethylammonium chloride pH 2)–acetonitrile (90:10, v/v). The mobile phase programme was 0–5 min mobile phase 1, 5–20 min mobile phase 2, 10 min reequilibration between runs. The mobile phase was maintained at a constant flow-rate of 1.0 ml min^{-1}. Detection was by UV at 260 ± 4 nm. Quantitation was by reference to replicate injections of standard in mobile phase.

Extraction and clean-up procedure

Thinly sliced cattle kidney (10 g), 1 *M* aqueous sodium carbonate (2 ml) and acetone (25 ml) were homogenised for 2 min, placed in an ultrasonic bath for 3 min and centrifuged for 5 min at 4200 *g*. The supernatant was transferred to a separating funnel and the extraction repeated with a further 25 ml of acetone, 2 ml of 1 *M* aqueous sodium carbonate and 8 ml of water. Chloroform (50 ml), saturated aqueous sodium chloride (20 ml) and 40% aqueous sodium hydroxide (2 ml) were added to the combined supernatants, and the resultant mixture was shaken for 1 min. The lower organic layer was filtered through anhydrous sodium sulphate and phase-separation paper and evaporated to dryness under reduced pressure at 50°C. The residue was redissolved in methanol–(0.01 *M* sodium trifluoroacetate–0.01 *M* sodium acetate pH 7) (80:20, v/v) (3 × 3 ml), centrifuged for 5 min at 1860 *g* and the supernatant transferred to a prewashed Bond-Elut CBA (carboxylic acid, weak cation exchange) cartridge. The solid residue from the centrifugation was carefully washed with methanol–(0.01 *M* sodium trifluoroacetate–0.01 *M* sodium acetate buffer pH 7) (80:20, v/v) (1 ml) and the supernatant transferred to the cartridge. The cartridge was washed with methanol (4 ml) and elut-

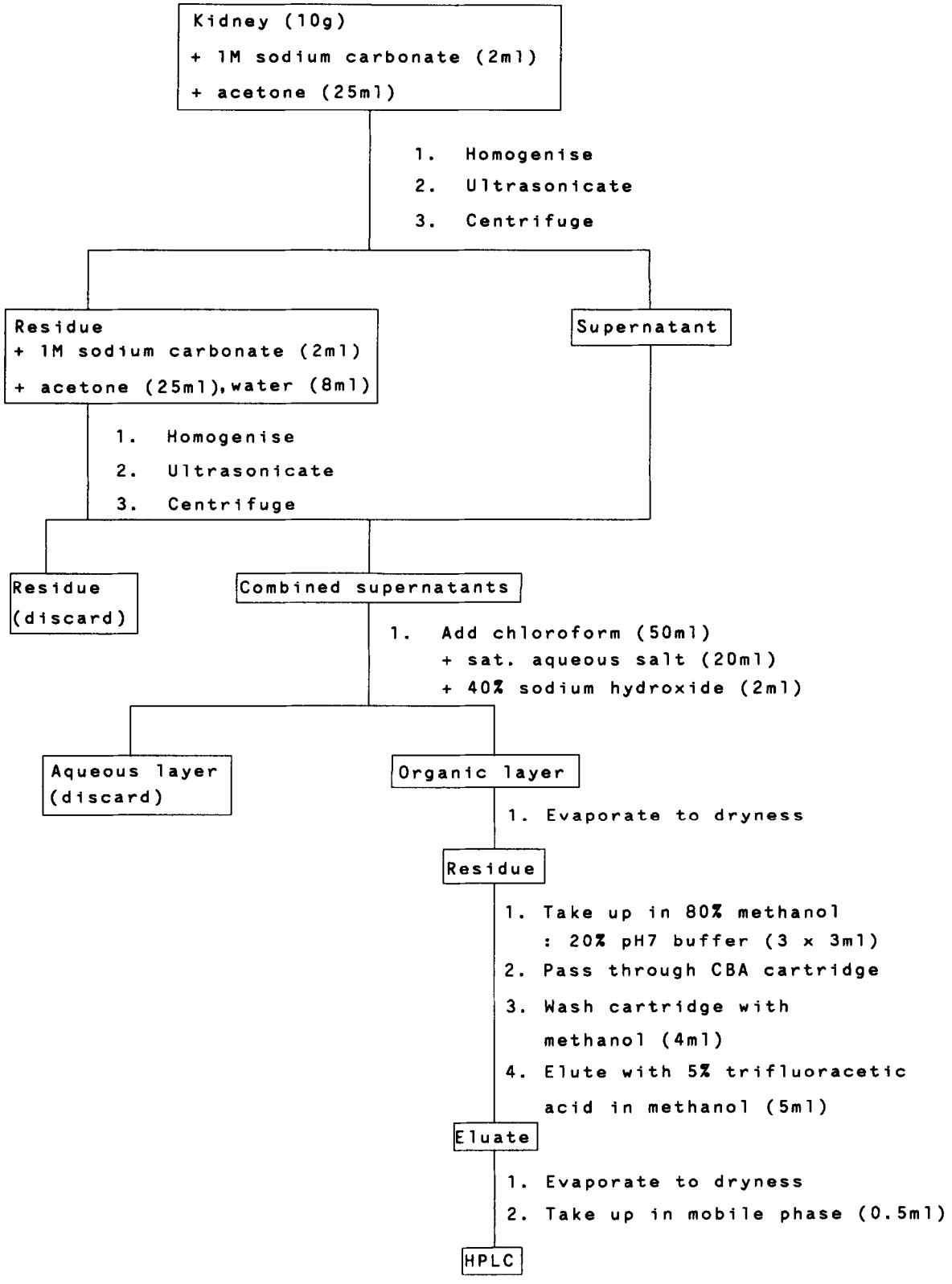

Fig. 2. Schematic representation of method.

TABLE I

RECOVERY OF IMIDOCARB FROM CATTLE KIDNEY SPIKED AT 0.01 AND 0.05 mg kg^{-1}

Batch	0.01 mg/kg			0.05 mg/kg		
	Recovery (mean ± S.D.) (%)	C.V. (%)	*n*	Recovery (mean ± S.D.) (%)	C.V. (%)	*n*
1	82.7 ± 2.0	2.4	4	79.9 ± 7.2	9.0	6
2	75.2 ± 7.1	9.5	6	77.6 ± 8.5	10.9	6
3	73.0 ± 5.7	7.8	6	81.6 ± 4.8	5.9	4
4				72.2 ± 10.7	14.8	6
Overall	76.3 ± 6.9	9.0	16	77.5 ± 9.0	11.6	22

ed with 5% trifluoroacetic acid in methanol (5 ml). The eluate was evaporated to dryness under reduced pressure at 50°C. The residue was redissolved in HPLC mobile phase 1 (0.5 ml) by vortex-mixing for 15 s and ultrasonication for 3 min, centrifuged for 5 min at 1860 *g* and filtered using a 0.45-μm syringe filter. A schematic representation of the method is shown at Fig. 2.

Protocol

Samples were analysed in batches consisting of four to six spikes and two blanks. Spiking was carried out by the addition of 0.5 ml standard at the appropriate level in acetone onto tissue and allowing the acetone to evaporate before commencing the analysis.

RESULTS AND DISCUSSION

Method validation

The method was validated at the 0.05 and 0.01 mg kg^{-1} levels. At the 0.05 mg kg^{-1} level, overall

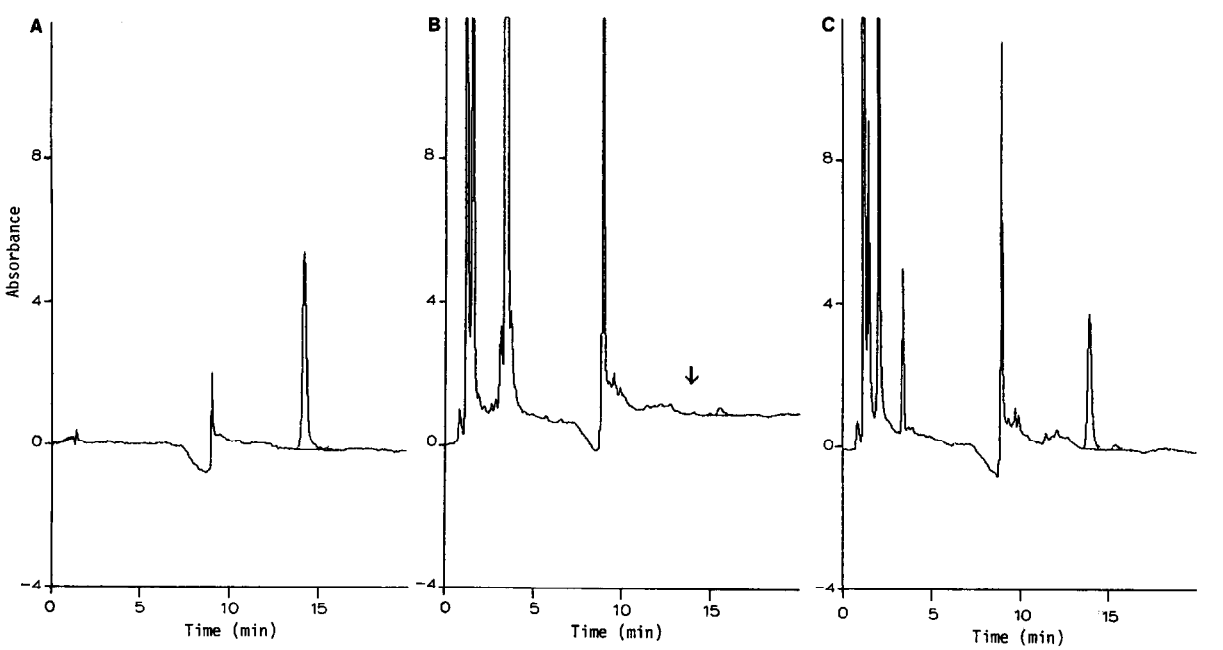

Fig. 3. Chromatograms of (A) standard imidocarb (1.0 μg ml^{-1}), (B) blank cattle kidney and (C) blank cattle kidney spiked at 0.05 mg kg^{-1} (scale in ma.u.f.s.).

recovery was 77.5 ± 9.0% (coefficient of variation, C.V., 11.6%) (Table I). At the 0.001 mg kg^{-1} level, overall recovery was 76.3% ± 6.9% (Table I). Typical chromatograms of standard, kidney blank and blank kidney spiked at the 0.05 mg kg^{-1} level are shown in Fig. 3. The limit of detection was estimated to be 0.001–0.002 mg kg^{-1}.

By increasing the injection volume to 100 μl, it was possible to monitor at the 0.001 mg kg^{-1} level. However, there is a significant retention time shift between standard and tissue spike with the increased volume. Recovery at this level was 67.5 ± 9.6% (C.V. 14.3%).

High-performance liquid chromatography

It was found that imidocarb is retained on reversed-phase columns at neutral pH and can be eluted efficiently by reducing the pH to 2. The reason for the strong retention of imidocarb at neutral pH is not totally clear but may be due in part to weak cation-exchange interactions with the acidic silica support which are absent at low pH by protonation of the surface. Since pH 2 is at the extreme edge of the normal working range of silica-based columns, the use of alternative columns was also examined. Both polymer (Polymer Labs. PLRP-S) and porous graphitic carbon (Shandon Hypercarb) columns gave poor chromatography. Phase Separations Spherisorb S3W-PC18 has a quoted stability range of pH 2–13 hence this column was chosen for further work. A number of buffers, such as citrate and phosphate, were tested for their suitability for the two mobile phases. Problems with retention time consistency and peak splitting were encountered. An acetate–trifluoroacetate buffer was found to give satisfactory results. The addition of 0.01 *M* tetramethylammonium chloride to mobile phase 2 was found to give sharper peaks, presumably due to blocking of residual silanol groups. Initially both mobile phase 1 and mobile phase 2 were maintained in the proportion of 85% buffer to 15% modifier (acetonitrile). It was subsequently found that this proportion of buffer to modifier in mobile phase 2 gave rise to an interference which made quantitation difficult at lower levels (<0.050 mg/kg). A satisfactory separation was obtained by reducing the modifier content of mobile phase 2 to 10%.

Examination of the diode-array spectrum of imidocarb showed that it had a single useful UV maximum at approximately 240 nm. Monitoring at 260 nm resulted in a 20% reduction in sensitivity, but also reduced interferences. The standard curve for imidocarb was linear from 0 to 500 ng on-column with a correlation coefficient of 0.9991.

Standard imidocarb was found to be stable in mobile phase 1 for greater than one month at room temperature and at 4°C.

Extraction and clean-up

Optimum extraction conditions had previously been found to be acetone in the presence of sodium carbonate as base [1]. This extraction procedure was followed with minor modifications. It was found that imidocarb is retained on C_{18} phase in the presence of 1:1 chloroform–acetone but is eluted using 5% trifluoroacetic acid in methanol. In the presence of tissue residues, it was found that imidocarb was not retained on C_{18} cartridges.

Examination of the structure of imidocarb indicates that cation exchange could be the method of choice for clean-up from tissue residues. Imidocarb is very strongly retained on SCX (strong cation-exchange) phase, failing to elute in up to 25% ammonia solution (specific gravity 0.88) in methanol. Imidocarb is retained on CBA (weak cation-exchange) phase in the presence of 1:1 chloroform–acetone (up to 100 ml) or methanol (up to 4 ml), and is eluted using 5% trifluoroacetic acid in methanol. Elution of imidocarb is by protonation of the cation-exchange surface rather than by deprotonation of the cation by base. In the presence of tissue components, retention and elution characteristics were similar (unlike C_{18} phase). Since, with pure standards, C_{18} phase appears to act in a similar manner to CBA phase, it may be that retention is also by a cation-exchange mechanism rather than by the normal reversed-phase mechanism.

The aqueous acetone extract was found to

cause flow problems during clean-up on CBA cartridges. Drop in analyte recovery was alsó noted. Recovery of analyte was improved by partitioning the extract between saturated aqueous salt and chloroform and evaporating the organic layer to dryness.

Initially, variable recovery was noted when methanol was used to transfer the residue to the SPE cartridge. Consistent recovery was achieved by dissolving the residue in methanol containing 20% buffer at pH 7. The use of a small amount of buffer at neutral pH ensures that the cartridges are within the pH range at which cation exchange can occur. Insoluble residue was removed by centrifugation prior to SPE clean-up to prevent clogging of the column. Silanization of glassware at the points in the method where the extract was taken to dryness was also found to increase recovery.

REFERENCES

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