

Thin layer chromatographic investigation of some veterinary drugs

Z.S. Hamad and B.M. Yahya

Department of Pharmacology, College of Pharmacy, University of Mosul, Mosul, Iraq

Abstract

A simple technique, using solvent extraction and thin layer chromatography for the detection and separation of a number of commonly prescribed veterinary drugs was studied. Blood and urine have been considered as possible biological samples taken from the animals. The extraction of each sample was done according to the type of the drug as it is acidic or basic, then the extract was spotted on TLC plate by capillary tube after lining the starting line first. Separations of the components of the samples were developed after running the plate in a tight lid tank containing solvent (mobile phase). The identification of separated spots were carried out after lining the end line for the solvent front then by spraying the plate with mercurous nitrate reagent for acidic drugs and acidic iodoplatinate or potassium permanganate reagent for basic drugs. The rate of flow value (R_f) for each compound was measured and the color of spots were determined. This work provides a simple and rapid qualitative technique for the detection of veterinary drugs used at therapeutic doses and in overdosage.

Keywords: Thin layer chromatography; Veterinary drugs; Drug analysis.

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التحري عن بعض الأدوية البيطرية بواسطة إستشراب الطبقة الرقيقة

زينة سظام حمد و باسل محمد يحيى

فرع الأدوية، كلية الصيدلة، جامعة الموصل، الموصل، العراق

الخلاصة

وصفت هذه الدراسة طريقة بسيطة بعد عملية الإستخلاص إستعمال طريقة إستشراب الطبقة الرقيقة لكشف وفصل عدد من الادوية البيطرية الشائعة الإستعمال. يعتبر الدم و الادرار افضل عينات تؤخذ من الحيوانات تستعمل للكشف و التحليل عن وجود الادوية. حيث تم استخلاص هذه العينات بطريقتين إحداهما خاصة بالادوية الحامضية والاخرة خاصة بالادوية القاعدية وتم وضع هذه العينات على الطبقة الرقيقة باستعمال الانابيبب الشعيرية بعد تحديد خط الابتداء ثم وضعت في اواني محكمة الغطاء تحوي على (الطور المتحرك) الخاص بكل النوعين من الادوية، بعد فترة زمنية قصيرة سوف تنفصل المادة الموجودة في العينة مع تحرك المحلول الى اعلى الطبقة و يحدد خط الانتهاء لجريان المحلول المتحرك وترش الطبقة الرقيقة بالمحاليل الكاشفة الخاصة بكل نوع من الادوية لتحديد مكان البقعة ثم تقاس قيمة R_f لها ويحدد لون كل عينة. هذه الدراسة أعطت طريقة نوعية سهلة وسريعة للكشف عن مجموعة من الادوية البيطرية بالجرع العلاجية والجرع المفرطة.

Introduction

Animal toxicoses are a frequent presenting complaint in veterinary emergency clinics and general practice (1). Over the years, biological fluid samples have been used for drug analyses (2). Identification of the drug is a prerequisite for optimal treatment (3).

The use of thin layer chromatography (TLC) for the separation and identification of compounds is one of the earliest chromatographic techniques (4). It has been

increased over the past few years for drug analyses, owing to the short development time of modern plates (5). Less than one hour is usually enough to separate and identify very small amounts of a specific drug. A second advantage is the high reproducibility obtained with this technique using reproducibly coated and conditioned plates with accurate sample application technique (6,7).

This work was carried out to investigate the possibility of detection and separation of a number of a commonly

prescribed veterinary drugs which may also be used in overdose.

Materials and methods

Adult albino rats were used in this work that have been taken from animal house of the College of Veterinary Medicine, University of Mosul.

Three rats were used for each drug in this study, one ml of blood and urine was collected from each animal after

administration of three doses of each drug, the doses represent the therapeutic dose (12) and duplicate (over dosage) given to the animals as in the following schedule.

Then blood or urine sample was divided into two parts, one part was extracted for acidic drugs and the other part for basic drugs.

Drug	Doses mg/kg	Route of administration	Time of sampling blood urine	
Diphenhydramine	5,15,30	i.m.	after 30 min	after 24 h
Diazepam	5,10,20	i.m.	"	"
Dexamethasone	1,2,4	i.m.	"	"
Metoclopramide	25,50,100	i.m.	"	"
Codeine	10,20,40	s.c.	"	"
Atropine	2,4,8	i.m.	"	"
Promethazine	5,10,20	i.m.	"	"
Albendazole	15,30,60	orally	2h	"
Chlorpheniramine	10,20,40	i.m.	30 min	"
Diclofenac sodium	1,2,4	i.m.	"	"

For acidic drugs

Hydrochloric acid (0.25 ml, 2 N) was added to 1ml blood or urine in a conical glass tube followed by 5ml of ether. The contents of the tube were then vortexed mixed for 30 seconds. The organic layer (4 ml) was transferred to a clean conical tube and evaporated to dryness under a stream of air in a water bath. The residue was redissolved in 50 μ l ether and 40 μ l was spotted onto the thin layer chromatographic plate using a capillary tube under a hot air stream.

The plates were thin layer plastic sheets of the nonfluorescent type (silicagel-60, 10x20 cm, Merk, Daratadt/ Germany). The plates were developed in glass tanks at 20-25°C with acetone: chloroform (1:9). This mixture should be prepared fresh on alternate days and the tank should be equilibrated for at least 45 min. The plates were developed to a height of 10 cm and air dried for a few minutes prior spraying by mercurous nitrate reagent for location of the spots.

For basic drugs

Ammonium hydroxide (0.25 ml, 2 N) was added to 1ml blood or urine in a conical glass tube followed by 5 ml of chloroform. The contents of the tube were then vortexed for 30 seconds. 4ml of chloroform was taken into a clean tube and evaporated to dryness under a stream of air, and the residue was redissolved in 50 μ l chloroform and 40 μ l was spotted onto the thin layer plate.

The plates were developed with strong ammonia solution: methanol (1.5:100). After the plates were dried, they sprayed either by acidic iodoplatinate or potassium permanganate. Authentic drug samples were spotted on to the thin layer plate and were run with each series of unknown sample (5).

Rate of flow values (R_f)

The basic measurement in TLC is the R_f value (5). The R_f value is constant for a substance in a particular chromatography solvent system used. R_f is a measure of the distance traveled by the compound from the origin (starting line) divided by the distance traveled by the solvent from the origin on the TLC plate. The range of R_f value obtained is from 0 to 1.

Generally, mobile phases have been developed which ensure that a particular drug will have a quite different R_f value in one system compared with other. By other means, for extra confirmation of identity of a compound, more than one solvent system may be used and also different types of spray reagents may also be used for localization of the spots (4,5).

Results

No endogenous substances from blood or urine were found to interfere with the method described above.

Table (1) shows the rate of flow (R_F value) and the colour of the spot. These parameters (R_F value and colour of the spot) are important in diagnosing the unknown spots which may be revealed from any biological fluid sample (4,5).

Drug	R_f value			Colour of spot
	Authentic standard	Blood extract	Urine extract	
Diphenhydramine	0.54	0.54	0.55	Brownish
Diazepam	0.73	0.73	0.73*	Intense brown
Dexamethasone	0.9	0.91	0.91	Bright yellow
Metoclopramide	0.4	0.4	0.39*	Yellowish brown
Codeine	0.34	0.33*	0.34	Intense blue
Atropine	0.17	0.17*	0.18	faint brown
Promethazine	0.5	0.5*	0.5	Yellowish brown
Albendazole	0.85	0.83*	0.84	Yellow
Chlorpheniramine	0.27	0.27*	0.27	Intense brown
Diclofenac sodium	0.76	0.77	0.77	Bright yellows

*The colour faint quickly.

The colour revealed depend on the doses of the drug (mentioned in the table (bold dose) each given to one rat of the 3 animals.

Discussion

When a clinician observes an animal with signs of poisoning, toxicological diseases which may be acute or chronic, and the clinical presentation will vary depending upon the route, extent, and frequency of exposure; the species involved; the time elapsed since the most recent exposure; and the route of exposure often influences the choice of specimens for confirmation (8).

Identifying an unknown substance in a biological fluid is a daunting task. It involves numerous steps: extraction, separation, and identification of the separated compound. Although the extraction of the acidic, neutral, and basic compounds is achieved by differential extraction, it is the separation and identification of the isolated compounds that pose the greatest challenge (3). In addition to these illicit compounds various drugs intended for medical use have been adopted into misuse (9).

The method described here is simple and rapid qualitative technique; a technician can carry out a check on a sample of blood or urine in 1 hr. The materials are readily available and inexpensive and need no specialized machine.

A simple analytical method for rapid screening of veterinary drugs is needed for use in areas that do not have fully equipped laboratories, it must be inexpensive, must require only limited facilities and minimum training, and must be capable of quickly analyzing the drugs, TLC method satisfies these requirements (10,11), and have been recommended as an effective quality screening method for the investigation of veterinary drugs.

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