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RESEARCH ARTICLE

DETECTION AND IDENTIFICATION OF XYLAZINE IN BLOOD SAMPLE OF CATTLE DURING THEIR ILLEGAL TRAFFICKING

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ABSTRACT

A case of utilization of xylazine to induce sedation in cattle to avoid their noise and movement for illegal trafficking was reported. A veterinary tranquilizer xylazine was detected by high performance thin layer chromatography followed by Gas Chromatography - Mass spectrometer. The blood sample of cattle and used syringes was received by the laboratory from the scene of crime by investigating agency. The extraction of drug from the biological samples has been done by using liquid-liquid extraction method. The methods strongly support the presence of xylazine in sample of question and give firm evidence for the material used for the purpose of crime.

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INTRODUCTION

In veterinary practice several sedatives such as Domodedane (Detomidine), Virbaxyl10%(Xylazine), Sedivet (Romifidine), Torbugesic (Butorphanol) etc. are in practice (http://www.elib4vet.com/vetdrugs/clinical_veterinary_drugs_list.htm). Xylazine is the common name for [2-(2.6-dimethylphenylamino)-4-H-5.6-dihydro- 1.3] thiazine, which is widely used in veterinary practice either alone as a tranquiliser or in blend with other drugs for sedation, analgesia, or general anaesthesia. There are ample of research papers available exploring numerous analytical methods for the detection of xylazinein plasma, urine, along with other biological fluids using liquid–liquid extraction (Moore, 2003; Hoffmann et al., 2001; Poklis et al., 1985; Stillwell et al., 2003 and Barroso1, 2007) and its pharmacokinetic study in animals (Garcia-Villar, 1981). Subject to kind of sedative used it is informal to trace or study the possible metabolites or drugs residues in the body fluids of specimen animal. However in forensic science trouble arises when scrutiny has to be done on biological specimen with unknown history for detection of possible intoxicant or causes. It is the puzzling task for the forensic expert to trace the exact material intricate with limited sample quantity.

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In present case the laboratory received the blood samples collected from body of big cattle which were illegally trafficked along with the used syringes to find out any intoxication used.

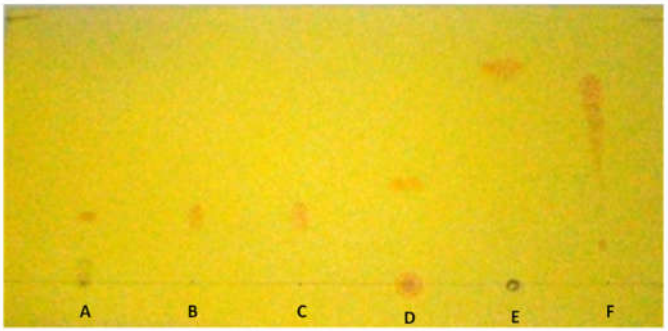
Experimental

By referring the fact that cattle's are under the influence of certain sedatives as their movement was arrested remarkably the rational way of liquid-liquid extraction were adopted for preparing sample for the analysis on HPTLC and GC-MS. Initially the scrutiny has been done on the available markers on HPTLC such as Ketamine, Xylazine, Aplrazolam, and Diazepam as all are known veterinary drugs. All Chemical and solvents used were of analytical grade. The reference xylazine, Alprazolam, Ketamine and Diazepam were obtained from local food and drug administration.

Extraction procedure

2 ml of blood sample was taken for the analysis in the test tube, 0.2 ml of concentrated hydrochloric acid was added, heated in autoclave at 15 psi for half an hour then cooled and P^H of sample was adjusted to 9.2 by adding aqueous ammonia solution. Solvent extraction of the sample was done by using chloroform and isopropyl alcohol (9:1) manually for 45 minute in separating funnel. After extraction organic phase was separated and evaporated by nitrogen and diluted to 1 ml with

methanol and filtered through 0.45 micron filter paper. In case of used syringes obtained from crime scenemethanol wash was taken and methanol was evaporated by nitrogen to concentrate the extract.



A: Blood Sample Extract(R_f 0.26),
B: Used Syringe extract (R_f 0.26),
C: Reference Xylazine,(R_f 0.26)
D: Reference Alprazolam (R_f 0.38),
E: Reference Diazepam(R_f 0.81),
F: Reference Ketamine (R_f 0.76)

Figure 1. HPTLC

High Performance Thin Layer Chromatography

HPTLC was performed on glass plate of size 20 cm X 20 cm pre-coated with 0.25 mm layer silica gel $^{60}F_{254}$. The plate was activated in oven at 110 ^{o}C for about 30 minutes then cooled at room temperature. The extracted sample and methanol wash of syringes was spotted followed by reference samples of xylazine, Aplrazolam, Diazepam and Ketamine with the help of capillaries on activated plate. The HPTLC plate was developed to a distance of 10 cm with Benzene: Acetonitrile: Methanol (8:1:1) as a mobile phase in a TLC chamber previously saturated with mobile phase. After development plate was removed and allowed to dry in air for 15 min and sprayed with Dragendorff's reagent. Rf vales of Orange spots were noted. (Fig. 1)

Gas Chromatography-Mass Spectrometry

Extracted samples prepared from the above mentioned procedure were injected to the GC-MS operated with following parameters and recorded the spectrum of reference xylazine followed by sample in question as shown in Figure 2.

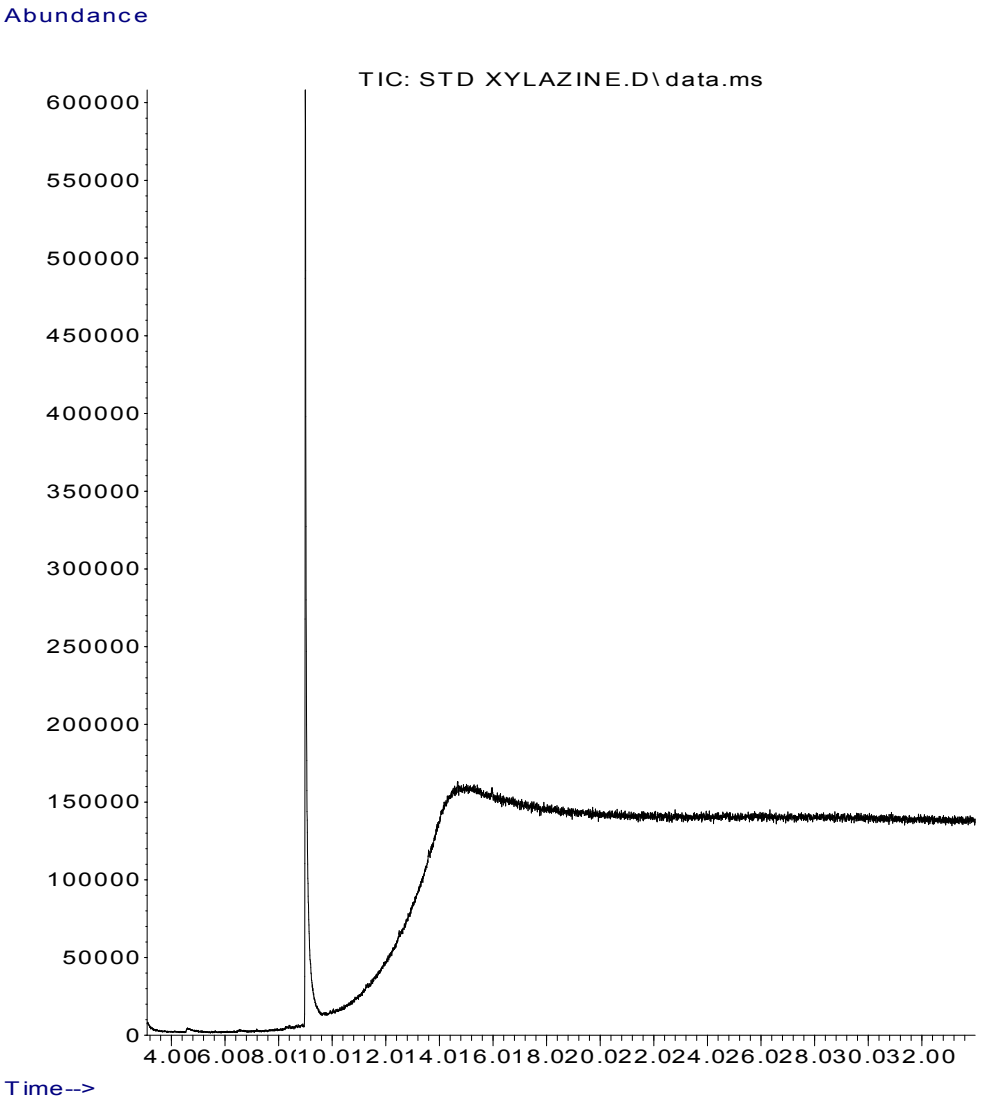


Figure 2. GC- graph and total ion chromatogram of reference Xylazine

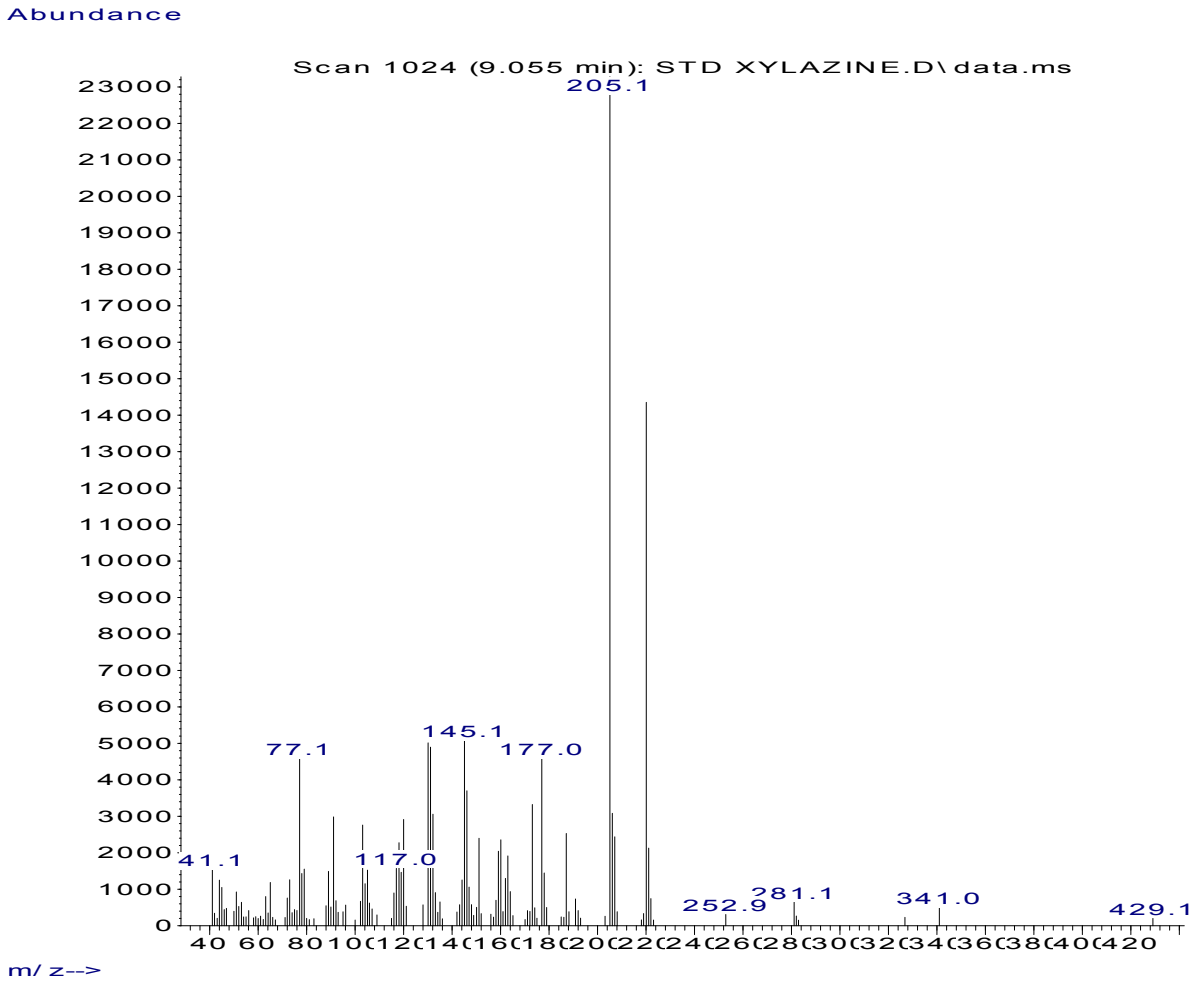
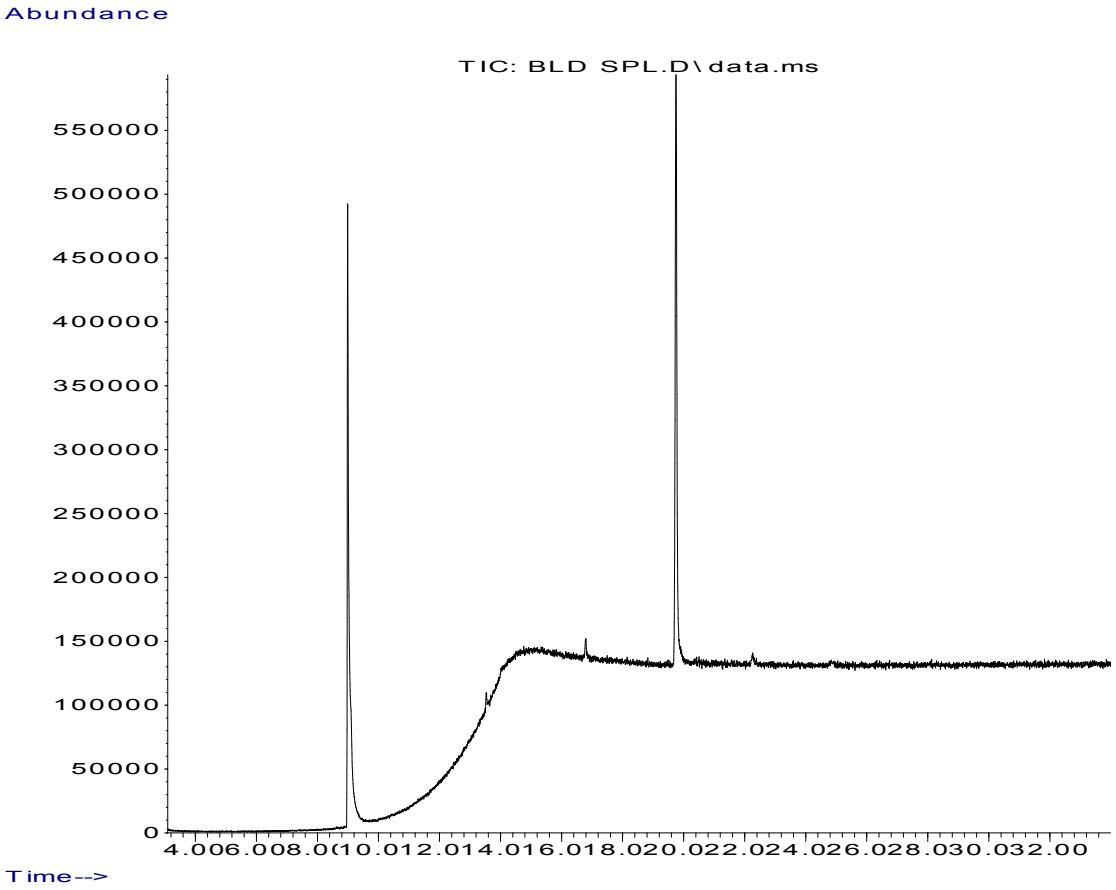
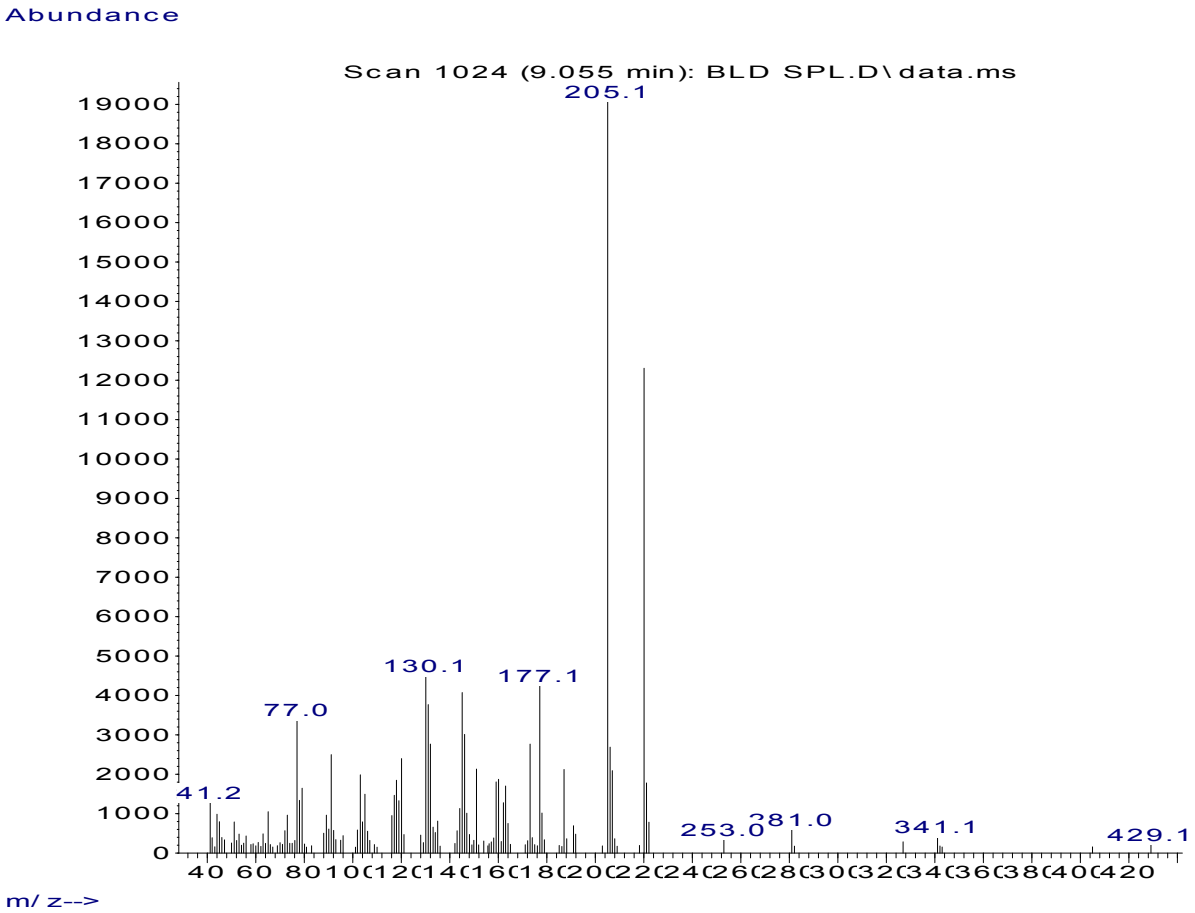


Fig. 2 (b). GC- graph and total ion chromatogram of Blood Sample extract





GC-MS Make and Model: Agilent GC-MS with triple axis detector.

GC-Conditions:

Oven Program: 150 °C for 1 min then 10 °C/min to 280 °C for 20 min
Run Time: 34 min,
Injection Volume and temperature: 1 µL, 250 ^{o}C
Mode: Split,
Split Ratio:10:1
Column: HP-5 MS 5% Phenyl Methyl Silox, 325 ^{o}C: 30 m x 250 µm x 0.25 µm

Mass Conditions:

Solvent Delay: 3.00 min,
Low Mass : 40.0,
High Mass: 550.0,
MS Source : 230 ^{o}C,
MS Quad: 150 ^{0}C

RESULT AND DISCUSSION

In HPTLC orange spot of xylazine, Alprazolam, Diazepam and Ketamine were obtained at R_f values 0.26, 0.38, 0.81 and 0.76respectively. The blood sample extract and methanol extract of used syringes showed same orange spot at R_f value of xylazine, thus providing positive evidence for presence of xylazine. In gas chromatography RT at 9.0 min and base peak in mass spectrum at 220 obtained for reference xylazine and suspected samples. Further the library search strongly supported the presence of xylazinein suspected samples. In conclusion the present case report illustrate detection and confirmation of xylazine in blood samples of cattle by using simple extraction procedure and very simple analytical techniques HPTLC and GC-MS. The methods strongly support the presence of xylazine in sample of question and give firm evidence for the material used for the purpose of crime.

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