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Detection of Counterfeit Drugs using Solid Phase Micro Extraction

Watson's Notes

Innovative Solutions for Difficult Problems

You have recently been in Afghanistan, I perceive"

With these words was born the most famous team in detective fiction; Sherlock Holmes and his trusted comrade and biographer, Dr. John H. Watson.

In the spirit of Watson, who chronicled the exploits of Holmes, we have created this newsletter named "Watson's Notes".

In the pages of "Watson's Notes", modern day scribes document the discoveries, unusual cases and other news of Investigative Science Incorporated, our scientific consulting firm in Burlington, Ontario, Canada.

Please contact us if you have comments, and please read on.

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Introduction

Solid Phase Micro-Extraction, or SPME, employs an adsorptive fibre which, when exposed to the headspace above an aqueous sample, will trap the volatile organic compounds given off by the sample. We have had good success with this technique for the detection of solvent vapours in drug and food preparations. It is extremely sensitive, and when combined with GC-mass spectrometry, offers the potential for a positive identification of any detected material.

In this study, we investigated the volatile organics in the headspace of a set of 60 veterinary drug preparations obtained locally in Kabul, Afghanistan. Some of the samples were thought ot be counterfeit.

Our approach was based on the premise that counterfeit goods may display a telltale signature of volatile material that is not present in the authentic goods. This volatile material could be a solvent used in illicit preparations or could simply be vapours present in the air where the material was processed. This may be expected to look different if the material was prepared in a clean factory versus a backyard, kitchen or field lab.

Methods

Samples were prepared by dispensing the drug products as received (in all but one case, the drugs were supplied as aqueous solutions) into a 20 mL vial fitted with a septum cap. A ten mL volume was used unless supplies were limited. The sample was heated to 70 °C and mixed with a magnetic stir bar during sampling. The headspace was sampled by exposing a Carboxen™ SPME fibre, injected through a septum cap, for 10 minutes at 70°C. The volatiles were detected by GC/MS specially equipped to accept the SPME fibres. The MDL was estimated to be about 1 ppb.

In order to evaluate the SPME results, we posed a series of questions arising from logical expectations concerning the data. These questions included:

1. Do any of the drug preparations contain volatile compounds that should not be present (eg poisons, carcinogens) or may indicate that the preparation was made under illicit circumstances?
2. When comparing products that are intended to be the same material from the same manufacturer, are the SPME volatile profiles the same?
3. Do different products display identical SPME volatiles profiles, indicating that they are, in fact, identical materials?

Presence of Toxic Volatiles

As shown in Table 1, we detected Class 1 (BP, to be avoided) and Class 2 (BP, to be limited) solvents in 27 of 60 preparations. Five of 60, or 8%, contained benzene; a Class 1 suspect carcinogen.

TABLE 1: VOCs Detected in Drug Products

Compound Detected	Drug Type	Number of Products
Benzene	Levamisole, Oxfendazole, Oxytetracycline	1, 3, 1
Chloroform	Albendazole	1
Toluene	Albendazole, Ivermectin, Oxfendazole, Oxytetracycline	2, 2, 2, 2
Chlorobenzene	Levamisole	2
Hexane	Oxytetracycilne	2
Pyridine	Pen/Strep	1
Xylene	Oxytetracycline	1
Styrene	Oxfendazole, Sulfadiazine	3, 1
Chlorophenols	Levamisole	3

Volatiles in products expected to be the same

The sample set contained 3 pairs of drugs, all supposedly prepared by Norbrook in County Doewn Ireland. As Figure 1 shows, however, products expected to be the same were actually visually different. First, the products behaved differently on storage. Sample 32K settled out on standing, while 8D did not.

ELEMENTARY MY DEAR WATSON

Uncertain About Heisenberg?:

! Werner Heisenberg was a German physicist known as the father of quantum mechanics. He is best known for his uncertainty principle, which states that one can not simultaneously know the velocity and position of an electron with certainty.

! There is a sign in Munich which states: "Heisenberg might have slept here"

! Earlier today Dr. Heisenberg stated unequivocally that he may or may not have been responsible for the Uncertainty Principle.

! The Heineken Uncertainty Principle: You can never be sure how many beers you had last night.

! Heisenberg is out for a drive when he's stopped by a traffic cop. The cop says: " Do you know how fast you were going? Heisenberg replies: "No, but I know exactly where I am".

Figure 1: Two Preparations of Norbrook Pen/Strep

As well, "injection" is misspellled on the 32K label. The SPME profiles for the two preparations were also widely different, as shown in Table 2.

TABLE 2: Comparison of the Headspace Volatiles of Two Preparations of Pen/Strep by SPME.

Volatile Compound	Sample 32K	Sample 8D
1,2 propane diol	ND	Main peak
Butyl acetate	Main peak	ND
1-butanol	present	ND
Methoxy propanol	ND	present
Methyl hexanone	ND	present
Benene acetic acid, butyl ester	present	ND
Glycerol	ND	present

ND: not detected

We expected that preparations reportedly made in the same factory in Ireland would have similar SPME profiles. In each of the three cases, the volatiles profiles were radically different, suggesting that they were not, in fact, made in the same place. The 32K Pen/Strep preparation displayed different physical characteristics as well as labelling anomalies. It was also found to contain no detectable levels of either drug.

The SPME profiles flagged the products as being different in each of these three cases. The drug assay results alone would have indicated that one of the products was suspect in only two of the cases. SPME is clearly an important addition to the toolkit of the forensic chemist.

Volatiles in products expected to be the different.

The results of this comparison were surprising. We observed that the profiles of several supposedly different products were, in fact, identical. In the first example, we compare two 1% Ivermectin injectable products obtained from the same supplier but bearing different product names and different batch numbers. The SPME volatiles profiles are shown in Figure 2.

Indectin (2K)

Ivotek (3K)

Figure 2: Comparison of Headspace Volatiles of Two Ivermectin Preps.

In this example, the SPME profiles are extremely similar, even down to the presence of several trace components such as styrene, toluene and ethyl butanoate. Methyl acetate is in the same proportion as ethyl acetate in both cases. Only the amount of 1,2-propane diol differs. The similarity of these two profiles suggests that the two products are the same material. HPLC anaysis of the active drug components supported this conclusion. In total, we detected 4 groups of drugs with virtually identical SPME profiles. In each case, each group of drugs having the same SPME profile was obtained from a unique local supplier. This strongly suggests that used containers from several suppliers were refilled with the same bulk material.

We conclude that SPME sampling of the headspace above veterinary drug products in water, combined with GC/MS analysis, is a powerful tool for the detection of counterfeit or suspicious drug products. The SPME approach is especially valuable for detecting counterfeit copies of known drugs, refilling of containers and adulteration with toxic solvents.

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