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Unexpected GC-MS Findings Following Immunoassay and TLC Screening in a Female Patient with Polysubstance Use: A Laboratory Case Report

Abstract – Polysubstance abuse is increasingly common and presents significant analytical challenges for toxicological laboratories. Standard screening methods such as immunoassay methods (for example urine dipstick test and enzyme modified immunoassay technique (EMIT)) and thin-layer chromatography (TLC) provide rapid detection but have limited specificity, particularly when structurally related compounds or adulterants are present. We report the case of a female patient with suspected substance abuse whose toxicological analysis revealed unexpected findings. Initial dipstick screening indicated polysubstance use. TLC supported some of the findings, particularly for cannabis; however, bands corresponding to other common drugs of abuse, including methamphetamine and morphine, were not observed. Therefore, gas chromatography–mass spectrometry (GC-MS) was performed as the final confirmatory analysis, which confirmed the absence of methamphetamine and morphine and instead detected pseudoephedrine and codeine. In addition, GC-MS analysis identified tramadol, another opioid, together with diazepam- and oxazepam-related benzophenone derivatives. The findings emphasize the need for confirmatory GC-MS in toxicological practice, ensuring precise identification of drugs and adulterants that may be overlooked by screening methods alone.

Keywords – *Polysubstance abuse, GC-MS, TLC, toxicology, drug testing*

1 INTRODUCTION

Polysubstance abuse is defined as the concurrent or sequential use of multiple psychoactive substances. It is a growing global health concern with significant clinical and forensic implications [1]. The complexity of such cases arises not only from the pharmacological interactions between drugs but also from the analytical challenges of detecting and identifying multiple substances in biological samples.

Routine toxicological screening often relies on immunoassays or thin-layer chromatography (TLC) due to their low cost and rapid turnaround time. However, these methods have their limitations including cross-reactivity with structurally similar substances and inability to detect adulterants [2]. On the other hand, gas chromatography–mass spectrometry (GC-MS) remains the gold standard for confirmatory drug testing, offering high specificity and the ability to identify a broad range of parent compounds and metabolites [3].

This report presents a case where TLC indicated polysubstance use, but subsequent GC-MS revealed a complex and unexpected spectrum of substances, including stimulants, opioids, antihistamines and benzophenone, an uncommon but notable adulterant. The case illustrates the analytical challenges faced by toxicology laboratories when dealing with polysubstance abuse

2 CASE REPORT

A 27-year-old Malay female with a history of cannabis use was referred for toxicological analysis after presenting with aggressive behaviour. Routine toxicology screening was requested to investigate possible polysubstance use.

2.1 Laboratory Findings

2.1.1 Dipstick Screening

Initial toxicological screening was performed using a commercial immunochromatographic urine test dipstick (Juschek®, Hangzhou AllTest Biotech, China) which provide a rapid, qualitative assessment of commonly abused substances in urine. The screening revealed positive results for

amphetamine-type stimulants (ATS), cannabis, opiates, and benzodiazepines, indicating the presence of multiple drug classes. While dipstick tests are convenient for preliminary detection, they are subject to cross-reactivity and limited specificity.

2.1.2 TLC Screening

Subsequently, the urine samples were analyzed using TLC as part of the toxicology evaluation. TLC for ATS (methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA)), tetrahydrocannabinol (THC) and opiates (morphine and codeine) were performed using 3 separate silica plates developed with individual mobile phases and visualizing reagents optimized for these drug classes (Figures 1–3).

The TLC analysis revealed clear positive bands for THC (Figure 1). Figure 2 shows that the methamphetamine band was not observed (spiked control Retardation factor (Rf) = 0.43)), and other ATS were also absent. However, a prominent reddish-brown spot at Rf = 0.37 was visible, which, based on previous TLC analyses, is likely to represent pseudoephedrine; this was later confirmed by GC-MS. Comparison with GC-MS results suggests that the blue band at a lower Rf correspond to a diphenhydramine metabolite, while the large reddish-brown spot at Rf = 0.6 is promethazine. Figure 3 shows morphine bands not seen (spiked control at Rf 0.31). However, a band at Rf 0.42, compatible with codeine was observed.

2.1.3 GC-MS Confirmatory Analysis

Confirmatory testing was performed using GC-MS which provided more specific identification of the compounds present in the urine sample. GC-MS analysis revealed a broad spectrum of substances spanning multiple pharmacological classes.

The sample underwent two separate liquid–liquid extractions prior to GC-MS: one under alkaline conditions and the other under acidic conditions.

For alkaline extractions, GC-MS analysis revealed the presence of a broad spectrum of substances spanning multiple pharmacological classes in the urine sample (Figure 4). A broad and tall peak for pseudoephedrine was seen at 6.232 minutes retention time. This tallies with TLC findings showing a massive spot compatible with pseudoephedrine on the ATS plate.

Norephedrine, its associated metabolite was also seen at earlier retention time. A small peak was identified by NIST library as oxazolidine pseudoephedrine. This is most likely an artifact caused by trace formaldehyde/acetaldehyde in ethyl acetate (used as mobile phase during TLC) reacting with pseudoephedrine to form an oxazolidine derivative.

A large peak for tramadol was seen at 10.839 minutes retention time. Major tramadol metabolites (O-desmethyltramadol and N-desmethyltramadol) were also seen on the chromatogram. A peak for codeine was seen at 13.121 minutes retention time. Sedating antihistamine, promethazine was also present.

On acidic extraction, additional compounds were also seen on the chromatogram, namely diazepam- and oxazepam-related benzophenone derivatives (2-methylamino 5-chloro benzophenone and 2-amino 5-chloro benzophenone respectively) (Figure 5). Other assorted substances seen include nicotine and its associated metabolite cotinine.

3 DISCUSSION

This case illustrates the limitations of dipsticks as an initial screening tool and TLC as a second detection method when analyzing complex drug mixtures. Although both dipsticks and TLC suggested polysubstance use, they could not distinguish between structurally similar compounds. GC-MS proved essential in providing unambiguous compound identification. Confirmatory GC-MS revealed a broad and unexpected spectrum of substances, including stimulants, opioids, sedating antihistamines, and diazepam- and oxazepam-related benzophenone derivatives (2-methylamino 5-chloro benzophenone and 2-amino 5-chloro benzophenone respectively).

THC, a compound present in cannabis, was detected by TLC in the present sample. The GC–MS method employed in this study was optimized for the detection of alkaline substances, including methamphetamine, morphine, and tramadol. Detection of THC would require a different extraction protocol and analytical approach and was therefore not performed. Nevertheless, the positive THC result obtained from the immunochromatographic dipstick, in conjunction with the confirmatory TLC findings, was considered sufficient, given the relatively low likelihood of false-positive results.

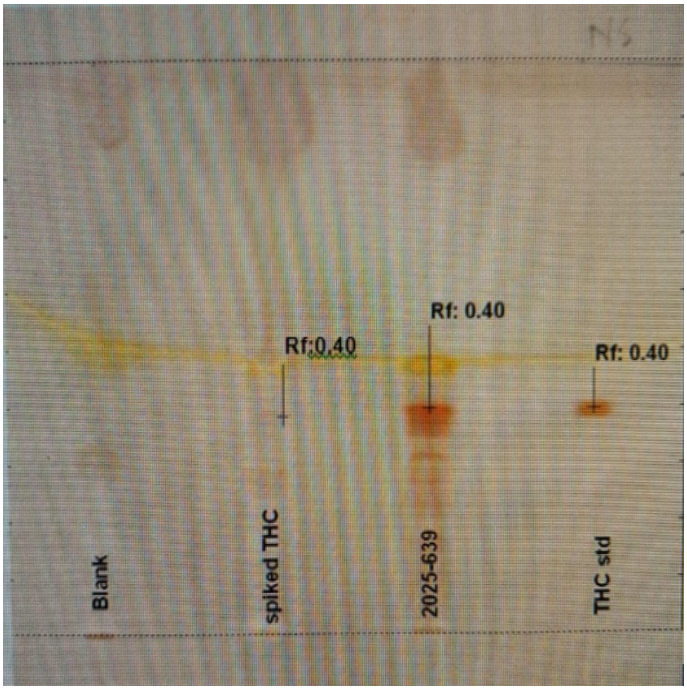

Figure 1. TLC plate used for screening of tetrahydrocannabinol (THC)

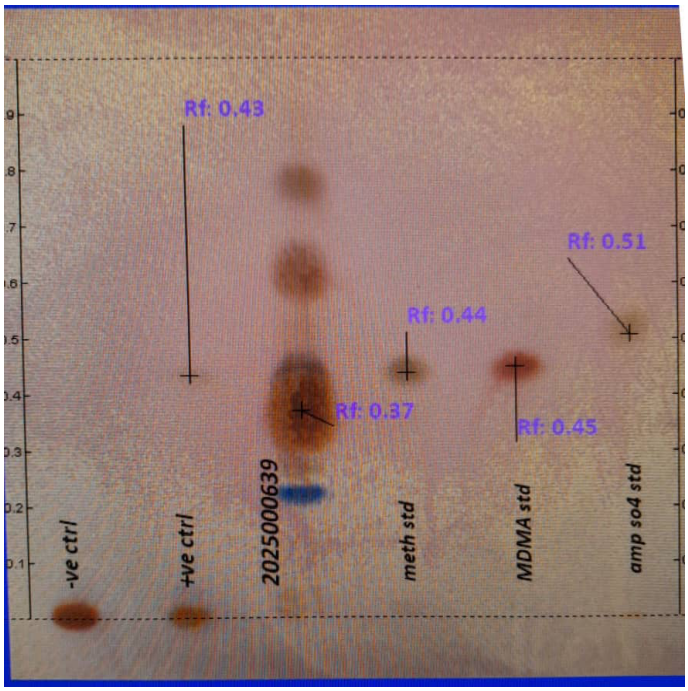

Figure 2. TLC plate used for ATS detection

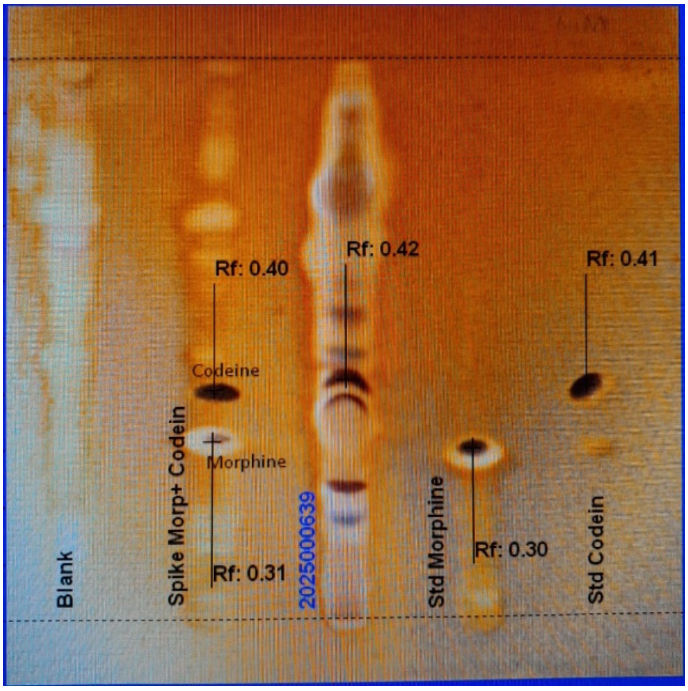

Figure 3. TLC plate used for screening of morphine and codeine

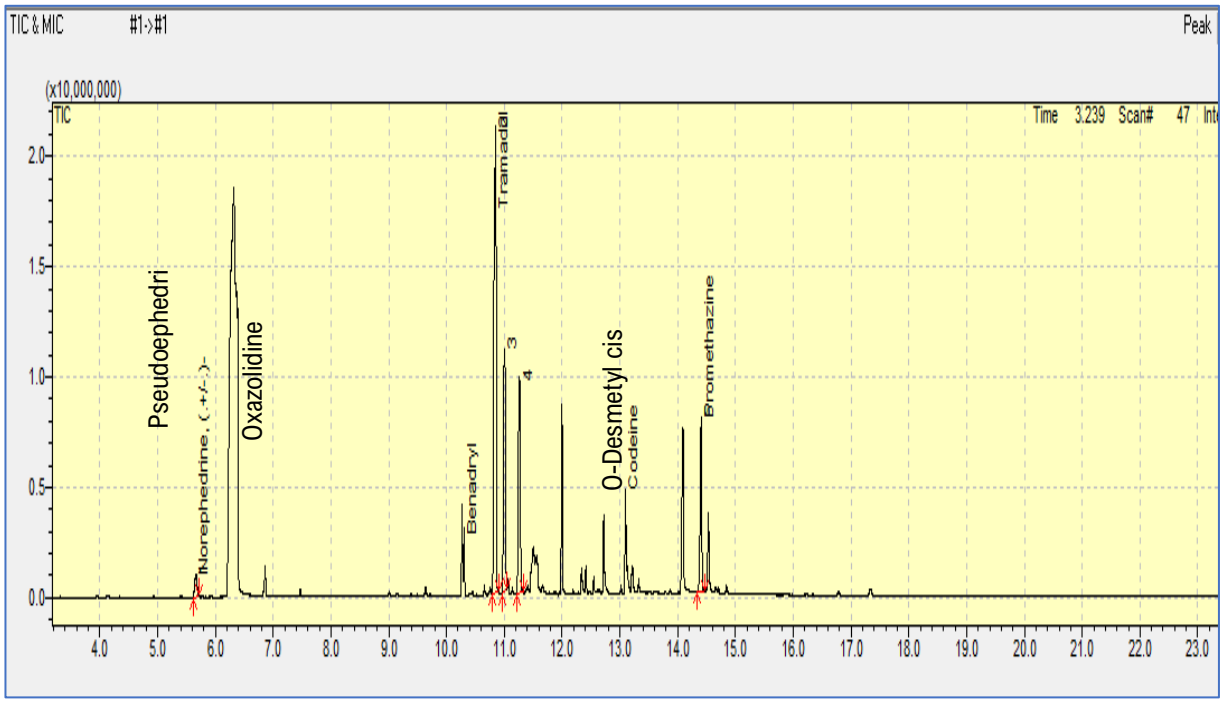

Figure 4. GC-MS chromatogram using alkaline extraction demonstrating the presence of multiple positive peaks corresponding to the detected substances

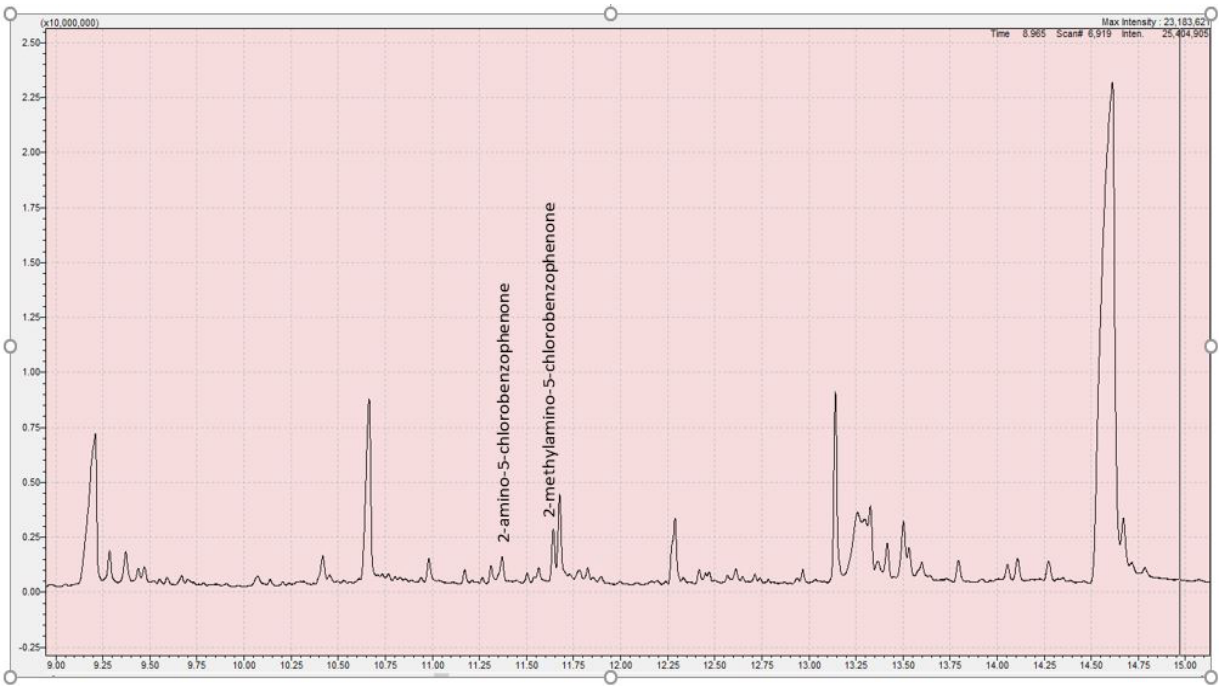

Figure 5. GC-MS chromatogram using acidic extraction, showing diazepam- and oxazepam-related benzophenone derivatives (2-methylamino 5-chloro benzophenone and 2-amino 5-chloro benzophenone respectively)

The observed discrepancy between the dipstick screening and GC–MS results can be attributed to the limited specificity of the immunochromatographic assay. Dipstick tests for ATS are designed as broad screening tools and do not differentiate between individual compounds (methamphetamine, amphetamine, MDMA). Moreover, they are prone to cross-reactivity with structurally related compounds such as pseudoephedrine and ephedrine, which may yield false-positive results. In contrast, GC–MS provides higher specificity and allows for definitive compound identification. In this study, GC–MS analysis did not detect any ATS but identified pseudoephedrine, indicating that the initial positive dipstick result was likely a false positive due to cross-reactivity.

The patient's history of cannabis use is clinically relevant, as cannabis is frequently co-used with other psychoactive substances to enhance euphoria, alleviate withdrawal symptoms, or modulate the effects of stimulants and opioids. Its presence provides important context for the complexity and polysubstance nature of the patient's drug-taking behaviour. This clinical history is further supported by the GC–MS findings, which demonstrated stimulants (ephedrine derivatives and nicotine), opioids (tramadol and codeine), and the sedating antihistamine promethazine. Together, these findings suggest a pattern of polysubstance use that may reflect attempts to enhance, offset, or counterbalance the pharmacological effects of co-administered substance.

Notably, promethazine is thought to enhance the euphoric effect of opioids and is therefore occasionally co-abused with opioids preparation containing codeine [8]. It is believed that the potentiation of opioids effects is mediated at least in part by delayed liver metabolism, causing its prolonged pharmacological effects.

The clinical presentation of aggressive behaviour may also be linked to the combined pharmacological actions of stimulants, opioids (including tramadol and codeine), sedating antihistamines, and possibly cannabis [4]. Stimulant exposure can increase agitation and impulsivity, opioids such as tramadol and codeine may contribute to mood instability, dysphoria, or withdrawal-related irritability, and antihistamines such as promethazine can occasionally cause paradoxical reactions including agitation [5-7].

Collectively, when combined with a background of cannabis use, these overlapping drug effects may amplify the risk of disinhibition and aggressive behaviour.

The detection of diazepam- and oxazepam-related benzophenone derivatives is particularly noteworthy, as it was not reported in the patient's drug history and is not a therapeutic agent. These compounds were only identified through GC-MS due to the superior sensitivity and specificity of this method as compared to the screening methods employed. Their detection can provide supportive evidence of benzodiazepine exposure. These compounds are formed from their parent benzodiazepines during sample preparation, particularly under acidic hydrolysis used to cleave glucuronide conjugates and improve benzodiazepine recovery for TLC analysis. Exposure to low pH conditions promote diazepine ring opening, resulting in the conversion of oxazepam to 2-amino-5-chlorobenzophenone and diazepam to 2-methylamino-5-chlorobenzophenone.

The benzophenone derivatives detected in this study are recognized degradation products of benzodiazepines rather than active substances or adulterants. These compounds can be formed during sample preparation, particularly under acidic hydrolysis conditions used to cleave glucuronide conjugates and improve benzodiazepine recovery for TLC analysis. In addition, low pH conditions can facilitate ring opening and further degradation of benzodiazepines. For example, oxazepam may be converted to 2-amino-5-chlorobenzophenonewhile diazepam may degrade to 2-methylamino-5-chlorobenzophenone [9]. Therefore, the presence of these benzophenone derivatives provides supportive evidence of prior benzodiazepines exposure and is consistent with the positive benzodiazepines dipstick screening result.

The wide range of pharmacological classes detected in this case reflects deliberate polysubstance use and highlights the analytical challenges and complexity toxicology interpretation when multiple substances co-exist. In such circumstances, reliable interpretation of toxicological findings relies not only on comprehensive confirmatory analyses, but also on clear understanding of strengths and limitations of the analytical methods employed.

Dipstick immunochromatographic tests are rapid, cost-effective screening tools but they are limited by low specificity and potential cross-reactivity, which may lead to false-positive or false-negative results. TLC provides a simple and inexpensive method for preliminary separation and identification of compounds; however, its sensitivity is limited and results may be subject to interpretational variability. In contrast, GC–MS is considered the gold standard due to its high sensitivity and specificity, allowing definitive identification of individual compounds based on their mass spectral profiles. Therefore, discrepancies between dipstick, TLC, and GC–MS results should be expected and reflect the inherent differences in analytical performance among these methods [10].

4 CONCLUSION

The present case illustrates the significant analytical challenges posed by polysubstance use, in which unexpected adulterants increase toxicological risk and lead to variable and unpredictable clinical presentations.

AUTHORSHIP CLARIFICATION

All authors meet the JBCS criteria for authorship and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was not required for this case report. Written informed consent was obtained from the patient.

CONSENT FOR PUBLICATION

All authors have read and approved the final manuscript and consent to its publication in this journal.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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