Short communication

Visualization of Amphetamine and Its Analogues in TLC

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Abstract

Derivatisation followed by iodine azide reaction was employed for detection of amphetamines and its analogues in TLC. The derivatisation reaction with phenyl isothiocyanate took place directly on the TLC plate before the developing step. Afterwards, the plate was sprayed with a mixture of sodium azide and starch solution and then exposed to iodine vapour. The obtained limits of detection were compared with other commonly visualization techniques: UV, iodine vapour, Marquis and Simon's reagents, ninhydrin, Fast Black K.

Keywords: TLC, iodine azide reaction, visualization of amphetamines;

1. Introduction

Amphetamine and its derivatives: methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine MDA, *p*-metoxyamphetamine (PMA), *p*-metoxymethamphetamine (PMMA) are stimulants of the central nervous system. All of the above substances are abused. EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) estimated total production of ATS (amphetamine type stimulants) in 2005 around 520 tons.[1]

Amphetamine (1-phenylpropan-2-amine) also known as 'speed' causes strong physical and mental stimulation. The abusers become alert, full of self-confidence, happy and talkative. They may feel like having more energy and being stronger. When the effect diminishes, their feelings evolve into anxiety, fatigue, disinterest or tiredness.[2] Amphetamine is produced mainly in Europe. The Leuckart reaction is the most common method used to obtain amphetamine, but in some cases reductive amination of 1-phenylpropan-2-one (BMK or P2P) is used. The other synthetic methods such as oxime or phenylnitropropene routes have not been put into practice in illegal production.[3,4]

Methamphetamine (*N*-methyl-1-phenyl-propan-2-amine) named 'crystal', 'crank', 'meth', 'Yaba' (tablets containing mainly methamphetamine and caffeine)[5,6] have a more pronounced stimulatory effect on the central nervous system than amphetamine. The high doses of this drug can result in effects such as hallucinations, paranoia and mania.[2] The main production centres are placed in East and South Asia.[1]

The drugs known as 'ecstasy', 'XTC', 'Beans' and 'Adam'[5] in 80% cases contain MDMA. However, their composition exhibits substantial variability. In tablets, the following substances: MDA, PMMA, PMA, amphetamine and methamphetamine, ephedrine, ketamine and caffeine were discovered beside MDMA.[7–11] Production of ecstasy tablets is concentrated in Europe but manufacture spreads to other parts of the world: North America, East and South-East Asia.[1] Reductive amination of 1-(3,4-methylenedioxyphenyl)-2-propanone (MDP-2-P or PMK) was found to be the most frequently used synthesis method of MDMA. Apart from this method the nitropropene route and safrole bromination were encountered. The Leuckart reaction to prepare MDMA was seldom used.[4,12–14] MDMA induce feelings such as euphoria, friendliness, closeness and empathy. But when used frequently, especially in high doses, it may cause cardiac arrhythmias, hyperthermia, lead to dysfunction of kidney, liver and brain damage.[8]

The analysis of seized drug tablets or powder is usually carried out using chromatographic methods: GC,[15–19] HPLC,[9,20] or TLC.[20,17,24] Other methods, e.g. near-infrared spectroscopy and Raman spectroscopy have also been applied.[21,22]

TLC is a low-cost and very versatile technique due to the availability of a wide range of possible developing systems. A great variety of visualizing reagents for detec-

tion of amphetamines in TLC was reported. Apart from observation of spots under UV light (254 nm), ninhydrin[23,24] and Marquis reagents[9,25] are most often applied. Also fluorescamine spray,[23] iodoplatinate solution were used.[15,23] Ojanpera et.al. detected amphetamines with Fast Black K (FBK) salt.[26]

In the present paper a new visualization and identification of selected drugs components (amphetamine, methamphetamine, MDMA, MDA, PMMA and PMA) is described. The procedure is based on iodine azide reaction. Its efficiency (detection limits) was compared with other visualization methods in which the following reagents were used: ninhydrin, FBK solutions, Marquis, Simon's and Ehrlich's reagents as well as iodine vapour were used.

The iodine azide reaction proceeds according to the following scheme:

$$I_2 + 2N_3^- \xrightarrow{\text{C=S inductor}} 2I^- + 3N_2.$$

This reaction had been successfully used for detection of amino acids and biogenic amines on TLC plate.[27,28] Woo et. al. had applied the iodine-azide reaction to detect amino acids in HPLC, as well.[29,30] Only sulphur(II) compounds induce the reaction. Because the analytes tested in the present work do not include sulphur atoms in their molecules, they need to be transformed into sulphur derivatives. Phenyl isothiocyanate (PTIC) was selected as the derivatisation agent. The derivatisation is a pre-chromatographic step and is performed directly on TLC plate. After development, the plate was sprayed with freshly prepared mixture of sodium azide (adjusted to proper pH) and 0.5% starch solution. Then the plate was exposed to iodine vapour. The plate background became violet-grey and the spots white.

2. Experimental

2.1. Solutions and Reagents

The following amines: MDMA (3,4-methylenedioxymethamphetamine), amphetamine and methamphetamine, MDA (3,4-methylenedioxyamphetamine), PMMA (*p*-methoxy-methamphetamine), PMA (*p*-methoxyamphetamine) used in the examination were synthesized in our laboratory. The examined drugs were dissolved in phosphate buffer pH = 7 (Merck. Germany).

Methanol, chloroform, 1,4-dioxane, *n*-hexane, 25% aqueous ammonia (all Merck, Germany, HPLC grade), toluene (Eurochem BGD, Poland, analytical grade) were used to prepare the mobile phases.

The spraying agent (iodine-azide reaction) was prepared by dissolving 2 g of sodium azide in 20 mL of distilled water and next adding 25 mL of 0.5% starch solution. Then, the pH of the mixture was adjusted (5.5–6.0) using 36% hydrochloride acid solution and diluted to 50 mL with distilled water.

The derivatisation solution was prepared by adding 20 μL of 98% phenyl isothiocyanate (PTIC) to 200 μL 2-propanol. The mixture was completed with 20 μL distilled water and 20 μL phosphate buffer pH 12 (3,58 g Na_2HPO_4 were dissolved in 80 mL water and the mixture was adjusted to pH = 12 with 1 mol L^{-1} NaOH and filled up by distilled water to 100 mL).

Marquis agent was prepared by mixing one part of 38% formaldehyde with five parts of 98% sulphuric acid (v/v).

Simon agent was composed of two solutions (A and B): 2% aqueous solution of sodium carbonate made solution A; solution B was prepared by dissolving 5 g of sodium nitroferricyanide in 100 mL of 10% acetaldehyde solution. The plate was first sprayed with solution A and then solution B.

Ehrlich agent was a 1% solution of *p*-dimethyloaminobenzaldehyde in mixture of hydrochloric acid and methanol (1:1 v/v).

FBK solution was prepared by dissolving 0.5 g of Fast Black K salt ($C_{14}H_{12}N_5O_4 \cdot 0.5ZnCl_4$) in 100 mL of water.

Ninhydrin solution was prepared by dissolving 0.2 g of ninhydrin in 100 mL of ethanol. All solution used in visualization of the spots on a chromatogram were prepared daily.

2.2. Procedure of Derivatisation of Amphetamine and Its Analogues

A portion (1 μL) of drug solution was spotted on TLC plate. Then, on the same place 1 μL derivatisation agent (PTIC) was applied. Next, the plate was put in the TLC chamber in order to complete the derivatisation process. After 20 min, the plate was developed with mixture of suitable solvents.

2.3. TLC Procedure

TLC silica gel $60F_{254}$ aluminium sheets (Merck, Germany; 10 × 10 cm, 0.2 mm thin layer) were used for determination of detection limits of amphetamine and its analogues. The plates were developed in the horizontal chamber (Camag, Switzerland). The developing distance was 8 cm. After derivatisation, the analytes were seperated using the following mobile phase: *n*-hexane, toluene, 1,4-dioxane (3: 3: 1 v/v/v) which made System I. In case the derivatisation was not carried out, another eluent was used: 1,4-dioxane, methanol, chloroform and 25% aqueous ammonia (6: 2: 2: 1 v/v/v/v) – System II. After development the plates were dried at 100 °C for 15 min. Then suitable spraying agents were applied.

2.4. Visualization of Spots on TLC Plates

The UV_{254} Procedure

After drying, the developed plates in System I and II were observed under UV lamp (254 nm). The substances quenched fluorescence of the plate background.

Table 1. Rf and detection limits (nmol per spot) of amphetamines detected as PTC-derivatives; mobile phase – System I.

Compounds	R_f	Visualization procedure		
		iodine-azide procedure	iodine vapour	UV_{254}
Amphetamine	0.44	0.19	0.19	0.19
Methamphetamine	0.45	0.17	0.17	0.17
MDMA	0.39	0.08	0.13	0.13
MDA	0.37	0.09	0.14	0.14
PMMA	0.42	0.15	0.15	0.15
PMA	0.40	0.14	0.14	0.14

The Iodine Procedure

After drying, the developed plates in System I and II were exposed to iodine vapour for 5 min. The drugs became visible as brown spots on yellow background of the plate.

The Iodine-Azide Procedure

After drying, the developed plates (System I) were sprayed with mixture containing sodium azide solution (spraying agent) and then exposed to iodine vapour for 5 s. Due to catalytic effect of the C=S bond on the iodine azide reaction, the examined compounds were visible as white spots on the violet-grey background. The colour of the plate has been stable for several minutes.

Application of Marquis Reagent

After drying, the developed plates (System II) were immersed in Marquis reagent and washed with distilled water. The spots were dyed blue-green.

Application of Simon and Ehrlich Reagents

After drying, the developed plates (System II) were sprayed with Simon or Ehrlich reagents and dried at 100 °C. The Simon reagent dyed the spots of drugs grey-green. In the case Ehrlich reagent was applied, the background became yellow and the spots white.

Application of FBK and Ninhydrin Solutions

After drying, the developed plates (in System II) were sprayed with ninhydrin or FBK solution and dried at temperature in range 100–110 °C. In the case ninhydrin reagent was used the purple-red spots on slight pink background were observed. The FBK dyed the spots red in the case of secondary amines and violet in the case of primary amines.

3. Results and Discussion

PTIC used as a derivatisation agent transforms amines to phenyl thiocarbamyl derivatives. This is why a different mobile phase has to be used.

In Tables 1 and 2, the values of R_f and detection limits for amphetamines are compared, corresponding to different visualization methods, after derivatisation of analytes.

The application of iodine azide reaction as the visualization method gave the best results. Apart from the low detection limits, an advantage of this visualization system over other methods (iodine vapour and UV_{254}), was the quality of the obtained chromatogram. The spots appeared more visible better shaped, observed as white spots on a violet-grey background, as compared with other examined methods.

Table 2. Rf and detection limits (nmol per spot) of amphetamines (without derivatisation; mobile phase – System II).

Compounds	R_f	Visualization methods						
		UV_{254}	iodine vapour	Ninhydrin	FBK	Marquis reagent	Simon's reagent	Ehrlich's reagent
Amphetamine	0.51	18.5	12.4	9.3	9.3	-	3.4	18.5
Methamphetamine	0.42	16.8	11.2	8.4	8.4	-	1.7	–
MDMA	0.41	8.6	8.6	6.5	6.5	2.6	1.3	–
MDA	0.52	9.3	9.3	7.0	7.0	2.8	2.8	13.4
PMMA	0.37	9.3	9.3	7.0	7.0	-	1.4	–
PMA	0.49	10.1	10.1	7.6	7.6	-	3.0	15.2

In Table 2, the detection limits obtained without derivatisation are presented. In some cases, limits of detection exceeded even by two orders the limits presented in Table 1, e.g. amphetamine detected under UV light or MDMA using ninhydrin as compared with iodine-azide procedure.

The iodine-azide procedure was successfully applied in determination of MDMA in samples that were synthesized in our laboratory according to various receipts which are used in clandestine laboratories.[12,14] The samples obtained from isosafrol and piperonal were studied. On TLC plate MDMA spot (R_f = 0.39) was accompanied by spots that came from derivatisation agent.

4. Conclusions

It has been shown that derivatisation of amphetamine and its analogues with phenyl isothiocyanate, a pre-chromatographic step in thin layer chromatography, makes it possible to decrease detection limits of all tested drugs as compared with other usually used methods of visualization of spots on TLC chromatograms, applied without derivatisation. Also, it was shown that visualization procedure based on iodine azide reaction belongs among the most sensitive methods of detection of amphetamines after their derivatisation. Though in the present paper only the most popular drugs were tested, it may turn out that the procedure proposed will be effective for other amphetamine analogues and other drugs that contain amine group as well.

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Povzetek

Za detekcijo amfetaminov in analogov pri tenkoplastni kromatografiji smo uporabili derivatizacijo, ki ji sledi reakcija z jodovim in azidom. Derivatizacijsko reakcijo s fenil izotiocianatom smo izvedli neposredno na plošči pred razvijanjem. Naknadno smo plošče napršili z mešanico natrijevega azida in raztopine škroba in jih nato izpostavili param joda. Meje določljivosti smo primerjali z drugimi običajnimi tehnikami vizualizacije: UV, jodove pare, reagenti po Marquisu and Simonu, ninhidrin, Fast Black K.