APPLICATION NOTE

Screening method for identification of forensic drugs

A-118.1

Keywords

Illicit drugs, psychotic substances, spice, stimulants, opioids, psychedelic drugs, cannabinoids, benzodiazepines

Introduction

The term “forensic” drug refers mainly to chemical or plant-derived substances that affect psychological, behavioral, or physical functions, and lead to varying degrees of dependence or addiction. Confiscated drugs have to be tested for their identity in a cost-efficient way in police labs worldwide. To check for identity by HPTLC, micro-chemical reactions (derivatizations) can be performed on the plate after development, evaluated within a short time, and at low costs. Further confirmation can be achieved by comparing UV and mass spectra of samples and references.

Scope

This method is suitable for screening of the most relevant forensic drugs. All substances are detectable by scanning densitometry with the TLC Scanner 4. A confirmation can be achieved by recorded UV spectra, mass spectra and/or derivatization of the substances. For mass detection, target zones are directly eluted from the HPTLC plate into the Waters ACQUITY QDa® using the CAMAG TLC-MS Interface 2. A fast screening method is shown below.

Required or recommended devices

Automatic TLC Sampler (ATS 4), Automatic Developing Chamber (ADC 2), Derivatizer, TLC Plate Heater 3, TLC Visualizer 2, *visionCATS*, TLC Scanner 4, UV Cabinet 4, TLC-MS Interface 2, Waters ACQUITY QDa Detector (Performance), Empower® or MassLynx® software

Samples

Real samples were provided by Institute of Forensic Medicine (Forensic Toxicology, Medical Center, University of Freiburg, Germany).
Powders were extracted in methanol or acetonitrile (1 mg/mL) by sonication. After centrifugation the supernatants were diluted (1:10 and 1:100) with the respective solvent.
For qualitative analysis of herbal mixtures (*e.g.* spice), samples were homogenized and extracted with methanol. For quantification, a triple extraction with methanol is recommended.

Standards

	Reference materials were provided by Lipomed AG (Arlesheim, Switzerland) as 1 mg/mL solutions in methanol or acetonitrile. Those were diluted 1:10 in the respective solvent. As a System Suitability Test (SST) 300 ng of Clonazepam and Flunitrazepam (each), 500 ng of DMT and APINACA (each), and 1000 ng of MDMA were applied.

Chromatography

Stationary Phase	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck) (for quantification better results are obtained after pre-washing the plates up to 80 mm with the developing solvent, followed by drying for 20 min at 120°C.
Sample application	Bandwise application with ATS 4, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume between 1-10 µL for sample and standard solutions.
Developing solvent	Toluene – acetone – diethylamine 85:10:5 (*v/v/v*)
Development	In the ADC 2 with chamber saturation (with filter paper) 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride.
Developing distance	70 mm (from the lower edge)
Plate drying	Drying 5 min in the ADC 2, heat plate for 3 min at 80°C on TLC Plate Heater to remove diethylamine
Documentation	With the TLC Visualizer 2: Before derivatization under white light, UV 254 and UV 366 nm, and after derivatization under white light and UV 366 nm
Densitometry	Densitometric analyses are performed at 200 nm (single-wavelength scan to localize zones for spectrum scan) and/or several wavelengths for quantification (multi-wavelength scan), slit dimension 5.0 x 0.2 mm, scanning speed 20 mm/s, spectra recording 190 to 450 nm.

Derivatization	(1) Reagent name: van Urk Reagent preparation: Under ice cooling, in a 10 mL volumetric flask, carefully mix 4.5 mL of water with 1 mL of concentrated sulfuric acid. Allow the mixture to cool to room temperature and add 0.1 g of 4-(dimethylamino)-benzaldehyde. Fill up to 10 mL with water. Reagent Use: The plates are sprayed with 3 mL of van Urk reagent with the Derivatizer, red nozzle, spaying level 4. Then the plates are heated at 120°C on the TLC Plate Heater for 2 min and chromatograms are captured with the TLC Visualizer 2. After this, the plates a heated for another 8 min at 120°C and images are captured again. *Alternative:* (2) Reagent name: Marquis Add 8.5 mL methanol and 1.5 mL sulfuric acid. Allow the mixture to cool to room temperature, then add 0.5 mL formaldehyde. Reagent Use: The plates are sprayed with 3 mL of Marquis reagent with the Derivatizer, red nozzle, spaying level 5. Then the plates are heated at 120°C on the TLC Plate Heater for 10 min and chromatograms are captured with the TLC Visualizer 2.
MS confirmation	Prior to derivatization, the zones to be eluted are marked with a soft pencil under UV 254 nm using the UV Cabinet or TLC Visualizer 2. For non-UV-active compounds: standards or samples are applied twice. One part of the plate is derivatized for localizing the corresponding zones on the non-derivatized part of the plate. Target zones are directly eluted using the TLC-MS Interface 2 with oval elution head into the ACQUITY QDa Detector at a flow rate of 0.5 mL/min with methanol. For a full scan spectrum it is recommended to first elute a blank, which can be subtracted from the spectra of the target zones. For confirmation of substances between 50 and 500 ng per zone are required.
MS parameter	The ACQUITY QDa Detector is operated in ESI^+ and ESI^- mode using default parameters. The ESI capillary is set to 0.8 kV, cone voltage to 15 V, and desolvation temperature at 600 °C. A full scan mass spectrum between *m/z* 100 and 600 is acquired at a sampling rate of 10.0 points/sec (continuum). Data processing and evaluation of mass spectra are performed with Empower. For routine use in quality control Single Ion Recording (SIR) can be performed.

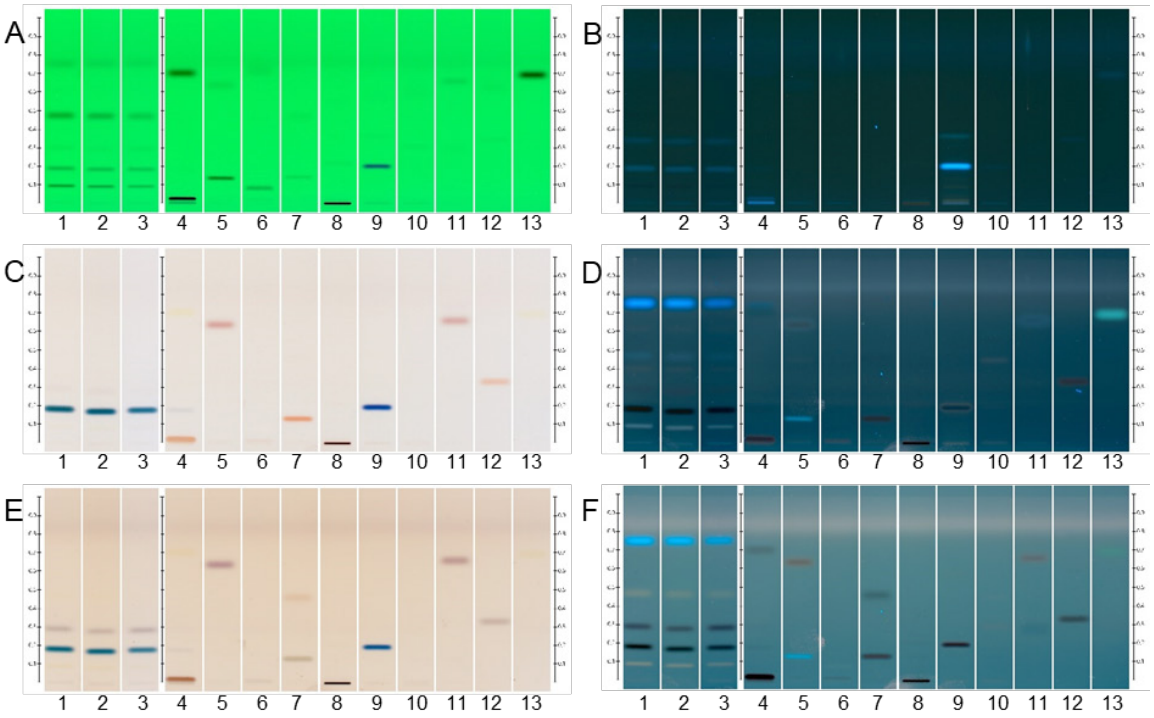
Results

System Suitability Test (SST) under UV 254 nm prior to derivatization:

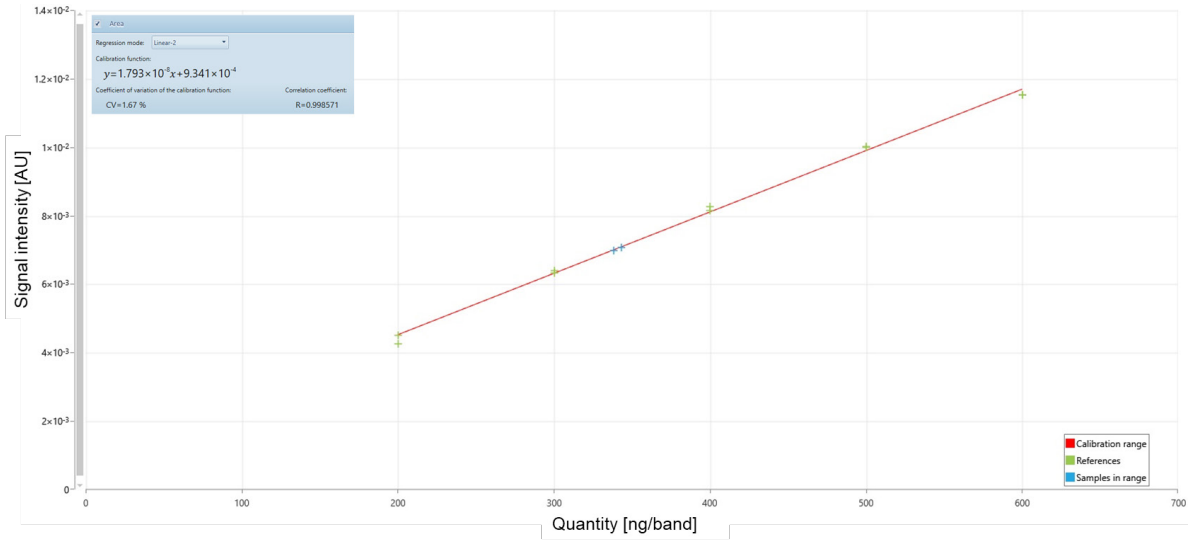
- Clonazepam shows a zone at R_F 0.09
- DMT shows a zone at R_F 0.18
- MDMA shows a zone at R_F 0.30
- Flunitrazepam shows a zone at R_F 0.48
- APINACA shows a zone at R_F 0.76

NOTE: The presented results are to be regarded as examples only!

HPTLC Chromatograms of forensic drug standards



HPTLC chromatograms under UV 254 nm (A), under UV 366 nm (B) prior to derivatization, under white light (C) and UV 366 nm (D) after derivatization with van Urk reagent (2 min heating), and under white light (E) and UV 366 nm (F) after derivatization with van Urk reagent (10 min total heating time); Tracks 1, 2, 3: SST; track 4: morphine, XLR-11, cathinone; track 5: alprazolam, THC; track 6: secobarbital, phencyclidine, methylphenidate; track 7: codeine, oxycodone; track 8: psilocin, ketamine; track 9: LSD, Fentanyl; track 10: metamphetamine, cocaine; track 11: 2C-B, CBD; track 12: heroin, methadone; track 13: amphetamine, JWH-018 (each on overspotted tracks in sequence with increasing R_F values)

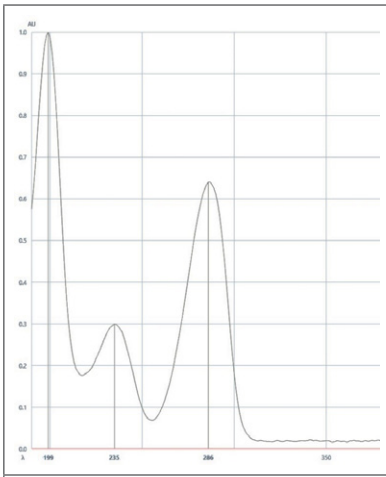


Calibration curve of cocaine (green standards and blue replicates of a cocaine sample), scanned at 234 nm, (linear working range between 200 and 600 ng)

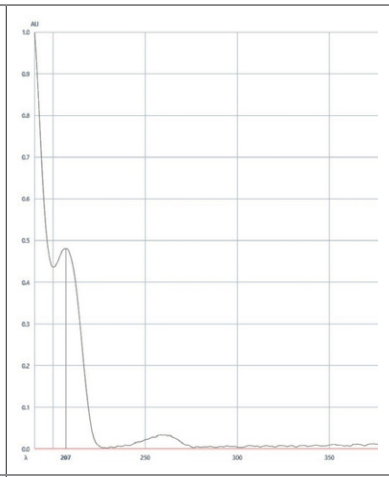
Table 1

Substance	R_F	UV 254	UV 366	van Urk (2 min, 20°C)		van Urk (10 min, 120°C)		Marquis (20 min, 120°C)		Absorption max. (nm)	*m/z* [M+H]⁺	*m/z* [M-H]⁻
				UV 366	White light	UV 366	White light	UV 366	White light			
Morphine	0.02	+	+	+	+	+	+	+	+	212	286	
Clonazepam	0.09	+	+	+	+	+	+	+		220		314
Secobarbital	0.09	+				+		+		239		237
Alprazolam	0.13	+		+		+		+		223	309	
Codeine	0.14	+		+	+	+	+	+	+	212	300	
DMT	0.18	+	+	+	+	+	+	+	+	222		187
Psilocin	0.21			+	+	+	+	+	+	224	205	
LSD	0.20			+		+	+	+	+	245	324	
MDMA	0.30	+				+	+	+	+	286	194	
2C-B	0.31					+		+	+	202	261	
Methamphetamine	0.32					+	+	+		207	150	
Heroin	0.34	+	+	+	+	+	+	+	+	206	370	
Amphetamine	0.34					+	+	+		206	136	
Cathinone	0.43							+		206	152	
Flunitrazepam	0.48	+	+	+		+	+	+		304	314	
Oxycodone	0.49	+				+	+	+	+	205	316	
Methylphenidate	0.50							+		205	234	
Ketamine	0.56									214	238	
Fentanyl	0.59					+	+	+		204	337	
Cocaine	0.61	+								234	304	
Δ-9-THC	0.64	+		+	+	+	+	+	+	211		313
CBD	0.66	+		+	+	+	+	+	+	211		313
Methadone	0.65	+						+		295	310	
JWH-018	0.71	+	+	+	+	+	+	+	+	218	342	
XLR-11	0.72	+	+	+	+	+	+	+	+	307	330	
Phencyclidine	0.73							+	+	210	244	
APINACA	0.76			+	+	+		+	+	211	365	

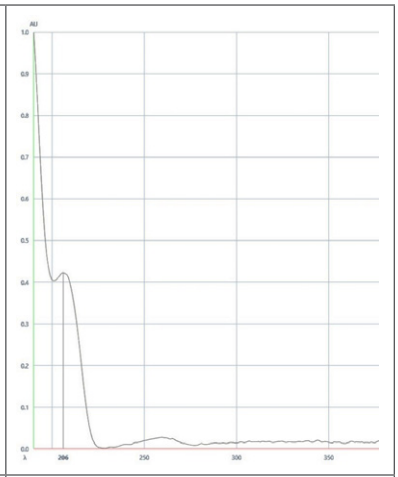
UV Spectra



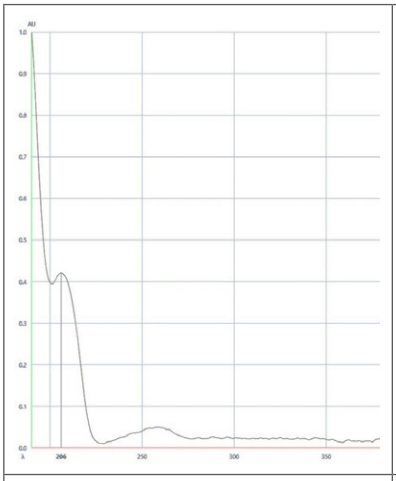
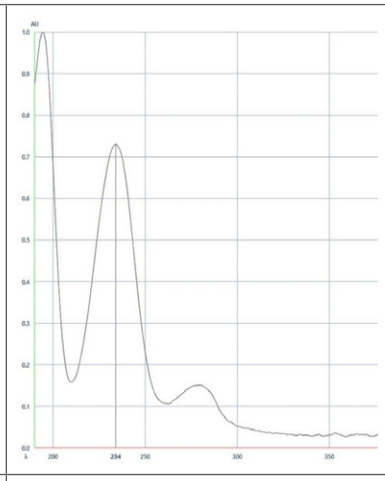
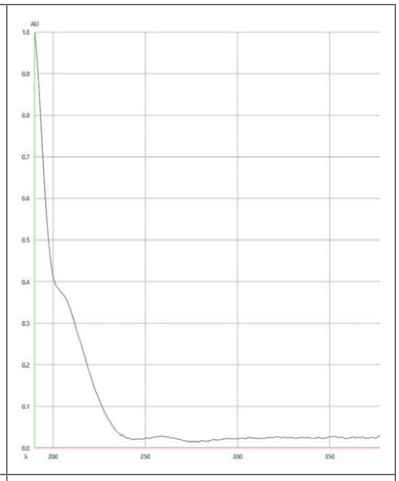
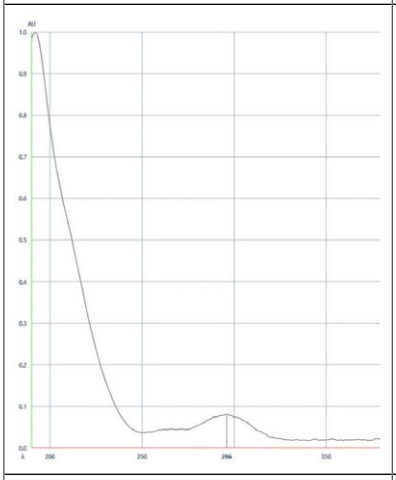
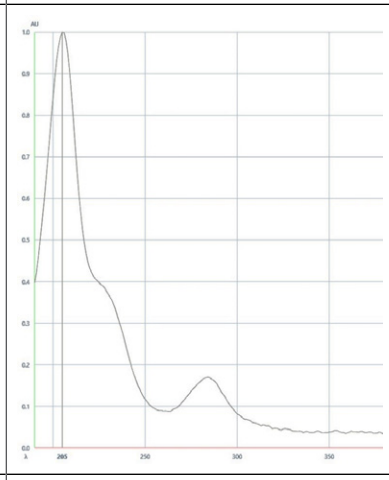
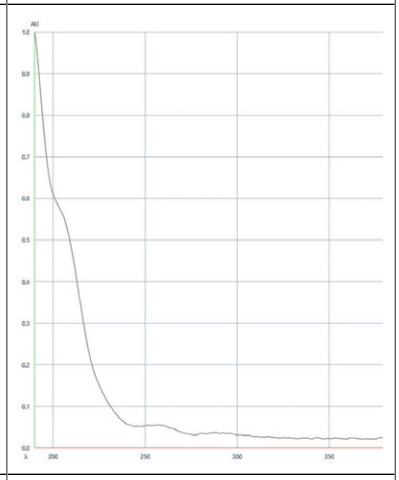
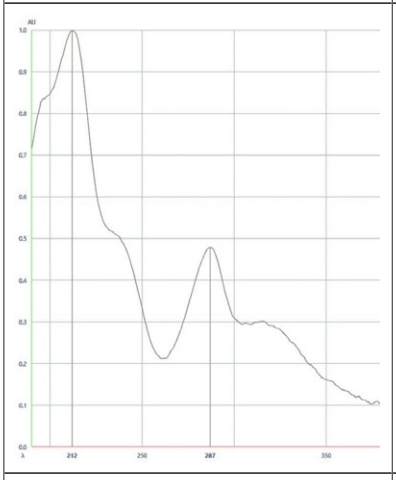
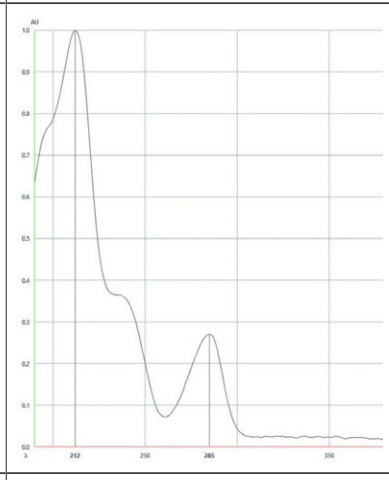
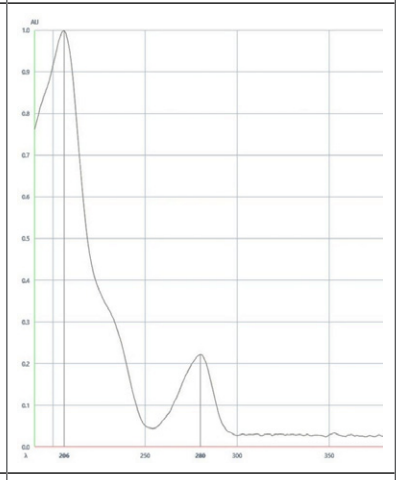
MDMA



Methamphetamine

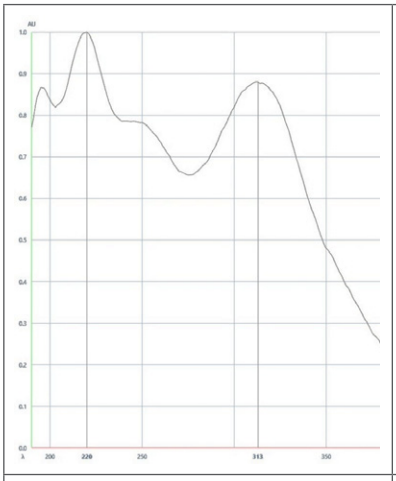
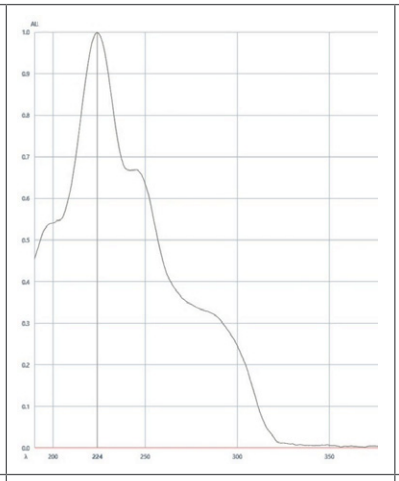
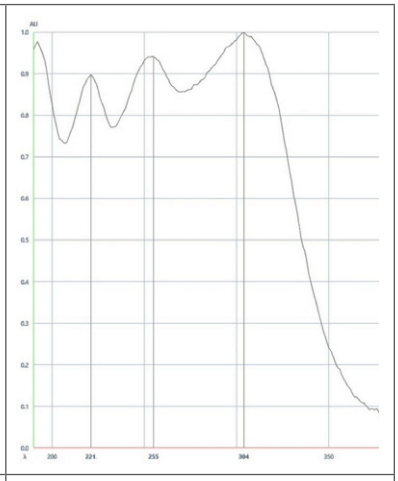
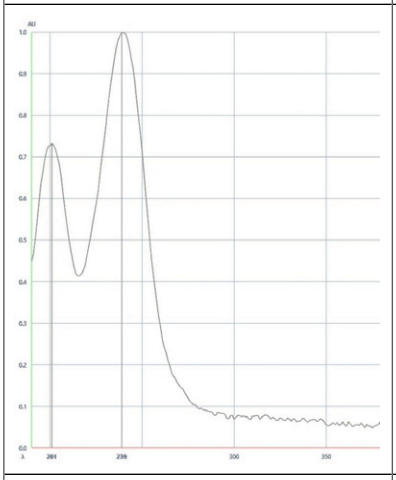
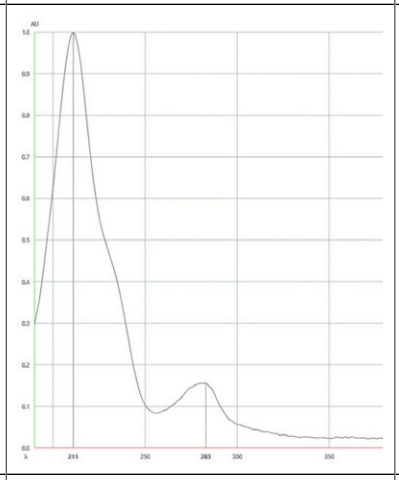
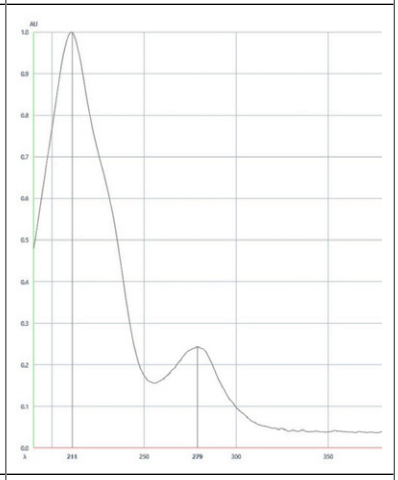


Amphetamine

		
Cathinone	Cocaine	Methylphenidate
		
Methadone	Oxycodone	Fentanyl
		
Morphine	Codeine	Heroin

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products!

		
Clonazepam	Alprazolam	Flunitrazepam
		
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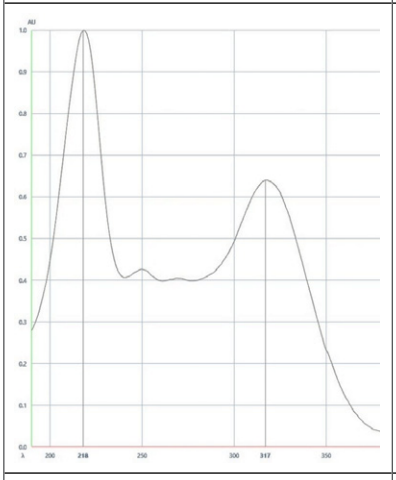
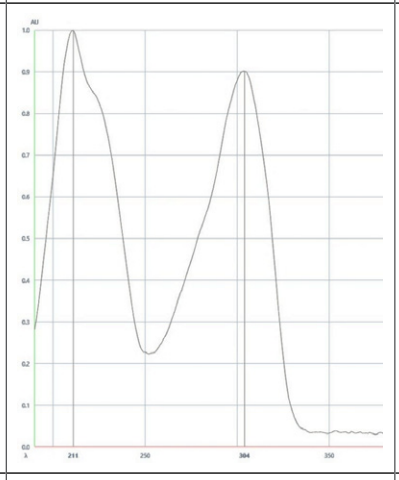
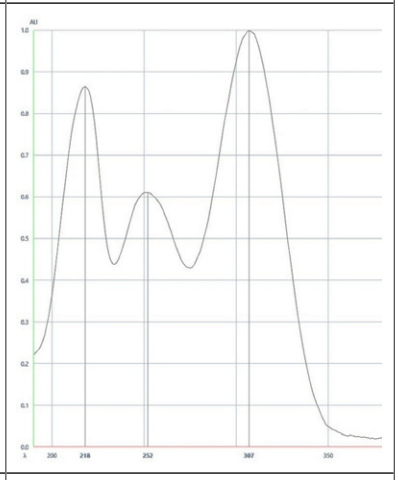
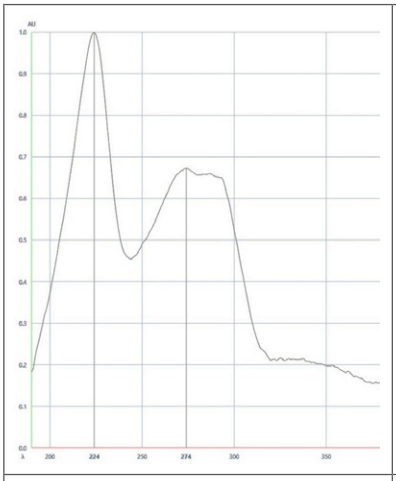
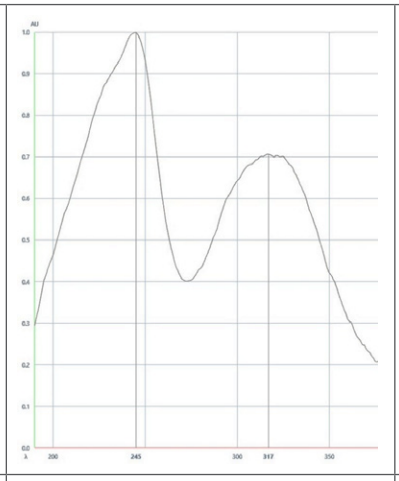
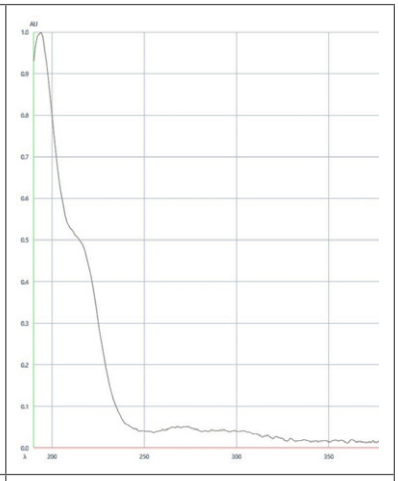
		
JHW-018	APINACA	XLR-11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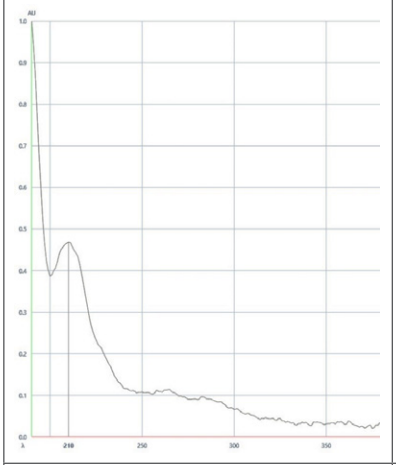	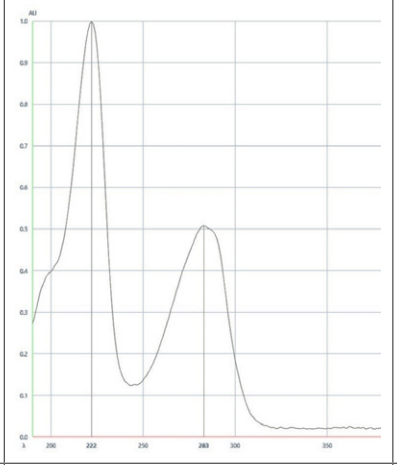	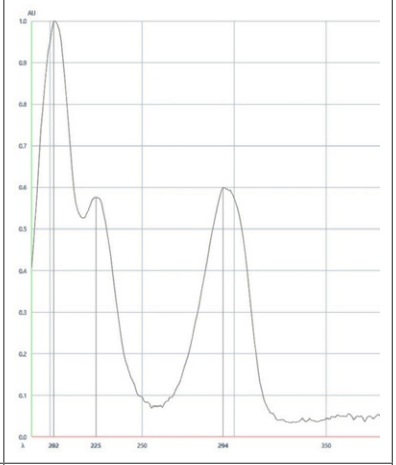
Phencyclidine	DMT	2C-B

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Literature

Bachelor Thesis Corinna Henninger (2018) Entwicklung eines Schnelltests zur Drogenidentifikation mittels HPTLC, University of Applied Sciences Offenburg