When Things Have to go Fast – Screening Drug Paraphernalia using DART-HRMS

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Introduction

What's the Project?

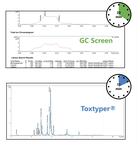
The recording of injected substances is mainly based on self-reported information from treatment registries and ad hoc surveys, which are often delayed and not analytically validated. Information about injecting individuals outside the healthcare system is also limited.

The ESCAPE (**E**uropean **S**yringe **C**ollection and **A**nalysis **P**roject **E**nterprise) project of the European Union Drugs Agency (EUDA) aims to close these gaps through timely, local chemical analysis of residual contents of used syringes. It enables monitoring local trends as well as multi drug use and adulteration, possibly leading to elevated risks of adverse health effects and overdose deaths.

In 2024, the ESCAPE network consisted of 16 participating countries, and syringes were collected from 77 collection sides across Europe.

What's the Analytical Bottle Neck?

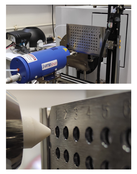
In forensic toxicology, screening for drug of abuse - whether in body fluids, drug paraphernalia, or substance samples - is typically performed using gas or liquid chromatography coupled with mass spectrometry. Depending on the method used, runtimes of these analyses usually vary between 10 and 25 minutes. Data analysis and reporting may also take some time. For quick and easy screening, these steps should be performed in as little time as possible and with a high degree of automation.



What's Direct Analysis in Real Time (DART)?

DART is an ionization technique that allows solid, liquid, and gaseous samples to be analyzed directly on a wide variety of surfaces at atmospheric pressure without any special preparation.

It has been demonstrated a suitable tool for the analysis of a variety of samples, especially in forensic chemistry. n recent years, it's applications in clinical or forensic toxicology have also increased. In combination with high resolution mass spectrometry (HRMS) this chromatography-free approach provides spectral information for targeted and untargeted screening within a very short analysis time of only 20 to 60 seconds.



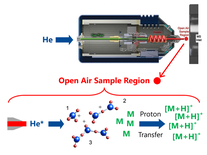
How does the Ionization Process Work?

I. **Ion Source:** An inert gas (e.g. He or N_2) is excited by an electrical discharge, creating metastable species

II. **Ionization Process:** These metastable species interact with atmospheric components, e.g. H_2O or O_2, to form reagent ions (1. Hydronium Ion, 2. Zundel Cation, 3. Eigen Caton)

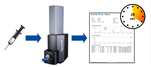
III. **Sample Interaction:** The reagent ions react with the surface molecules of the sample (solid, liquid, or gas) placed in the open gas stream, typically through proton transfer or Penning ionization

IV. **Analysis:** The newly formed ions are carried into the mass spectrometer for analysis



What's the Objective of This Project?

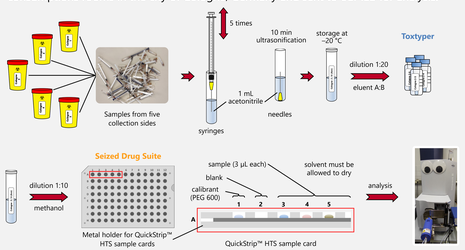
The aim of the project was to evaluate a rapid screening workflow for drugs of abuse and adulterants in used syringes using DART-HRMS.



Methods

Sample Collection and Preparation

Samples of this project were collected at 5 different harm-reduction services and drug consumptions rooms in the city of Cologne, Germany and sent to our lab for analysis.



Analytical Methods

Routine Toxtyper Screening

LC-System: Dionex UltiMate 3000 LC-System
Eluent A: H_2O, 2 mM HCO_2NH_4, 0.1% HCOOH, 1% acetinitrile
Eluent B: Acetonitrile, 2 mM HCO_2NH_4, 0.1% HCOOH, 1% H_2O
Column: Acclaim® RSLC 120 C18 2.2 µm 120A 2.1x100 mm
Gradient: 11 min gradient elution

MS-System: Bruker amaZon speed™ ion trap
Ion source: Apollo ESI source, positive mode
Scan mode: UltraScan (70 - 800 Da @ 32,500 Da/s)
MSn mode: AutoMSn (n = 3) with Scheduled Precursor List
Library: In-house generated library with 1,475 entries

Bruker Seized Drug Suite

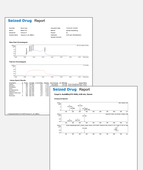
MS-System: Bruker impact II
Ion source: DART JumpShot® positive mode
Acquisition: ScanningLinear @ 0.5 mm/sec
Scan Range: 4.5 mm
Gas: Run Gas: Helium; Standby Gas: Nitrogen
Temperature: 275 °C
Scan mode: AutoMS/MS @ 9 Hz
Scan range: m/z 30 - 1000
Library: Seized Drug Suite (400 entries) and MMHW Library (Wiley-VCH; 5,000 entries)

Data Analysis

For both methods, data evaluation and reporting were performed fully automatically using identical scripts in DataAnalysis.

With **Toxtyper**, identification is performed by comparing retention times and MS^2/MS^3 spectra with an in-house spectrum library containing 1,475 entries.

The **Drug Screen Suite** identifies features found via the exact mass of the molecular ion and comparison of MS^2 spectra with the associated spectral library (400 entries) and the MMHW LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites (Wiley-VCH; 5,000 entries).



Runtime Comparison ...*because things have to go fast*

The total runtime of a batch consists of the method duration and the additional time required for injection (Toytyper) or movement of the QuickStrip™ HTS sample card (Seized Drug Suite). For LC-MSn analysis of drug paraphernalia, injection of an eluent blank after each sample is mandatory to check for possible carry-over.

	Method Runtime	Batch Runtime*	Consumables*
Toxtyper Screening		7176 min ≈ 5 d	1.6 L eluent A 1.0 L eluent B
Seized Drug Suite		340.1 min ≈ 5.7 h	≈ 370 L He

**calculated for 299 samples incl. blanks and calibration*

Results

Samples from the 2024 Campaign

	Syringes with	Syringes w/o	Needles	No of Samples
Side I	29	4	2	35
Side II	11	6	28	45
Side III	44	7	7	58
Side IV	21	12	28	61
Side V	46	34	20	100

The syringes from Side V originate from the Cologne city area and were collected in parks and public places. It is unclear how long they were exposed to the weather. As shown on the picture above, these samples were the most contaminated.

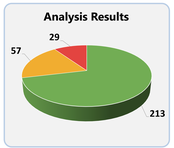
Data Evaluation and Method Comparison

The automatically generated analysis results of the **Toxtyper** and the **Seized Drug Suite** were checked and compared with each other. Each of the samples was assigned to one of three categories (see graph on the right):

Consistent results: Both methods identified the main active ingredient in the sample. Any degradation products and accompanying substances were not taken into account.

Low intensity results: Compounds not detected with DART-MS but low signal intensity in the LC-MSn run.

Inconsistent results: Main active ingredients were not identified by DART-HRMS.



Overall, consistent analysis results were obtained for **71.2%** of the samples.

Exemplary Analysis Results

Syringe Vision 01

Toxtyper	Int.	S/N	Seized Drug Suite	Int.
Cocaine	6.5e8	536	Cocaine	1.0e5
Benzoylecgonine	1.9e8	680	Benzoylecgonine	9.7e4
ADB-4en-PINACA	1.0e8	298	ADB-4en-PINACA	5.0e3
Ecgoninemethylester	6.5e6	39	Caffeine	3.1e4
Norcocaine	2.2e6	8	Paracetamol	8.5e5
Paracetamol	1.6e6	2		

- Cocaine was detected in the syringe
- The synthetic cannabinoid ADB-4en-PINACA was identified by both methods
- Toxtyper additionally detected Ecgoninemethylester (S/N 38) and Norcocaine (SN 8)
- Paracetamol showed a bad library match with both methods

Needle DKR 24

Toxtyper	Int.	S/N	Seized Drug Suite	Int.
Noscapine	5.9e8	1371	Caffeine	5.6e5
Caffeine	4.9e8	624	Paracetamol	2.1e5
6-Acetylmorphine	3.5e8	632	6-Acetylmorphine	1.4e5
Paracetamol	2.2e8	335	Noscapine	8.4e4
Heroin	9.7e7	348	Heroin	6.8e4
Papaverin	7.2e7	267	Papaverin	1.6e4
6-Acetylcodeine	5.9e7	176		
ADB-4en-Pinaca	1.6e7	41		

- Heroin, incl. poppy alkaloids, was detected in the needle
- Both methods idetified the common cutting agents caffeine and paracetamol
- Toxtyper additionally detected 6-Acetylcodein an additional heroin marker
- The synthetic cannabinoid ADB-4en-PINACA could not be identified with DART-MS, probably due to low concentration

Syringe Vision 4

Toxtyper	Int.	S/N	Seized Drug Suite	Int.
Cocaine	9.9e9	5036	Benzoylecgonine	5.7e4
Benzoylecgonine	2.5e9	5516	Cocaethylene	5.6e4
Noscapine	1.3e8	431	Cocaine	4.0e4
Norcocaine	1.2e8	273	Caffeine	7.1e3
6-Acetylmorphine	9.9e7	229	Ecgoninemethlester	5.0e3
Ecgoninemethlester	5.3e7	247	Norcocaine	4.2e3
Paracetamol	2.2e7	34		
Papaverin	2.0e7	64		
Caffeine	1.6e7	35		
6-Acetylcodeine	9.5e6	29		
Heroin	4.1e6	15		
Cocaethylene	1.6e6	3		

- Cocaine and degradation products / metabolites were detected by both methods
- Detection of cocaethylene (low LC-MSn intensity) by the Seized Drug Suite
- DART-HRMS did not detect heroin or any of the poppy alkaloids
- Although it is very frequently used as an adulterant, the detection of caffeine alone does not indicate the presence of heroin

Conclusions

ESCAPE Projekt

- As expected, heroin, cocaine or a combination of the two were the substances most frequently injected by this clientele. In addition, there were a small number of amphetamine, MDMA, methadone, and methylphenidate findings.
- The most surprising finding was the detection of the synthetic cannabinoid ADB-4en-PINACA in a total of nine samples from four different collection sides. According to other members of the ESCAPE network, there were reports of heroin laced with synthetic cannabinoids.
- Analysis of syringe residues offers an additional and complimentary view on the compounds injected within a certain community of drug users.

DART-HRMS Analysis of Syringes... *lessons learned in this first DART-MS project*

- The syringes sent in were very often contaminated with blood, which led to significantly dirtier extracts than initially thought.
- As it is completely unpredictable how much of a substance is still present in this type of sample material, one usually starts with higher dilution levels to avoid contamination, which may initially lead to negative results.
- The differing analysis results of substances with lower signal intensity or S/N in the LC-MSn run require a strategy for determining which dilutions should be used in future projects, e.g. difend parameters for reanalysis of a lower dilution step.
- Due to the different influences on the ionization yield and different matrix effects of the two ionization methods, comparisons between signal intensities can only be made to a limited extent.
- Although the LC-MSn analysis generally provided a more detailed picture of a sample, comparison with our routine screening method showed a high proportion of matching analytical results.

Did Things Go Fast?

- For this project, the overall analysis time was reduced by a factor of 21 compared to our routine screening.
- Depending on the analytical question, e.g. differentiation of isobaric compounds, strategies for re-analysis and use of a confirmatory analysis (e.g. LC-QTOF-MS) are recommended.
- The combination of DART with HRMS allows for a fast analysis of drug paraphernalia and a quick identification of compounds using targeted or un-targeted workflows. The workflow allows the use of third-party spectral libraries and the acquired HR-MS data can be used to identify unknowns.
- Depending on the analytical question, e.g. differentiation of isobaric compounds, a confirmatory analysis (e.g. LC-QTOF-MS) is recommended.
- Due to the unmatched speed, DART-HRMS is especially suited for rapid screening analysis, either as a stand-alone method or as guidance tool for subsequent in-depth analysis.

SCAN ME

Acknoledgements

We would like to thank the EUDA, especially the ESCAPE project staff, for their support.

Special thanks go to the employees of the following institutions and associations for collecting the syringes: Drug Consumption Room Gesundheitsamt Neumarkt, Association for Innovative Drug Self-Help Vision e.V. Köln, Emergency Shelter for Drug Addicts Cologne Main Station, SKM Köln e.V., Contact and Health Center Café Victoria, Drogenhilfe Köln gGmbH, and 'Projekt Feger' Collection of Syringe Paraphernalia in Public Spaces, SKM Köln e.V.