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Determining Psychoactive Drugs in Blood Plasma and Serum Using Automated SPE–LC–MS/MS

Oliver Lerch, Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany

The determination of psychoactive drugs is of great interest in the context of clinical and forensic toxicology. Easy and rugged determination of such compounds, down to the low ng/mL range from only 250 μL of blood plasma or serum, is described in this article. The comprehensively automated “multi method” is based on solid-phase extraction (SPE) coupled online to liquid chromatography with tandem mass spectrometry (LC–MS/MS). More than 60 compounds namely benzodiazepines, other sedatives, antidepressants, antipsychotics, methadone, and their relevant metabolites are included. The method has been fully validated for 52 compounds according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh). Some less relevant drugs and metabolites are included for semi-quantitative or qualitative determination.

Psychoactive drugs play a major role in many cases that are investigated in clinical or forensic toxicology laboratories. In addition to typical illicit drugs, such as, for example amphetamines, opioids, and cannabinoids, so-called new psychoactive
Substances (NPS) are often sold via the internet as legal highs, bath salts, or research chemicals. This trend has emerged in the last decade. Selling and purchasing these compounds is partly legal but legislative authorities have found ways of controlling these often dangerous substances by banning certain basic chemical structures.

In addition to illicit drugs, the abuse of prescription drugs dispensed for therapeutic purposes is an issue. Determining these compounds in blood plasma or serum and other biological matrices is the main topic of this article. Most prominent are the benzodiazepines, which are predominantly prescribed as sedatives, anxiolytics, and hypnotics and are the most widely used therapeutic drugs in psychiatry. Common for all benzodiazepines is the bicyclic structure consisting of a condensed diazepin- and benzene ring.

Some well-known active pharmaceutical ingredients are diazepam,lorazepam, and midazolam. Although benzodiazepines are less toxic than their predecessors, the barbiturates, intoxications can occur and can be fatal, especially in combination with other drugs such as alcohol and opioids. Long-term use can lead to physical addiction.

There are other sedatives that act in a similar way to benzodiazepines with a differing chemical structure referred to as non-benzodiazepines or sometimes colloquially “z-drugs” since many of their names begin with a “z”. Like benzodiazepines they include nitrogen-heterocyclic rings; typical representatives are zopiclone and zolpidem.

Beside tri- and tetracyclic antidepressants (TCA), the so-called selective serotonin noradrenalin reuptake inhibitors (SSNRI) are also prescribed as antidepressants. TCA are built of similar tri- or tetracyclic structures always containing a basic nitrogen atom, while SSNRI are a heterogeneous group of active pharmaceutical ingredients (API). Antipsychotics are drugs used for therapy of psychosis-like delusions, hallucinations, or paranoia. These are also a rather heterogeneous group of compounds.

In the context of prescription drugs methadone is also of interest. It is given in opioid replacement therapies under medical supervision. Co-consumption of sedatives and other prescription drugs is observed under replacement therapy in patients, to further dampen potential withdrawal symptoms. Co-consumption may need to be monitored to assess therapeutic success, which is one possible...
application of the analysis method described in this article.

Cases of driving under the influence of drugs (DUID) are another major area for which psychoactive compounds should be determined in blood. Driver impairment can be ascertained and causes of accidents can be elucidated. Moreover intoxications in clinical or forensic context can be discovered. When these substances are used in drug-facilitated crimes and, for example, given as “knockout drops” the victims’ blood must be examined.

A look into the scientific literature (1–7) reveals that benzodiazepines and other prescription drugs are normally extracted by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) from serum or plasma, oftentimes preceded by a protein precipitation step. Sometimes solid-supported LLE or solid-phase microextraction (SPME) are employed. Resulting extracts are routinely analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) or, after a derivatization step, by gas chromatography (GC)–MS/MS. With the availability of more sensitive mass spectrometers, protein precipitation without further cleanup or enrichment has been used for LC–MS/MS determination of the aforementioned compounds.

Figure 1: Example chromatograms of real samples at low analyte concentrations. Quantifier and qualifier MRM traces for: (a) Nordiazepam at 5.04 ng/mL (>LOQ), (b) Midazolam, 1-hydroxy- at 3.44 ng/mL (>LOD and <LOQ), and (c) Zopiclon at 1.25 ng/mL (>LOD and <LOQ).
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<th>Analyte</th>
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<th>ISTD for Quantitation</th>
<th>Validation</th>
<th>Calibration Range (ng/mL)</th>
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<td>Quantitative</td>
<td>2.5–100</td>
<td>1.3 / 2.5</td>
</tr>
<tr>
<td>Alprazolam, 1-hydroxy-</td>
<td>Benzodiazepine metabolite</td>
<td>Alprazolam-d5</td>
<td>Quantitative</td>
<td>2.5–100</td>
<td>1.3 / 2.5</td>
</tr>
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<td>Amisulprid</td>
<td>Antipsychotic</td>
<td>Nortriptylin-d3</td>
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<td>5–500</td>
<td>0.87 / 2.7</td>
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<td>TCA</td>
<td>Amitriptylin-d3</td>
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<td>5–500</td>
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<td>Aripiprazole</td>
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<td>Aripiprazol-d8</td>
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<td>2.3 / 4.4</td>
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<tr>
<td>Bromazepam</td>
<td>Benzodiazepine</td>
<td>Bromazepam-d4</td>
<td>Quantitative</td>
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<tr>
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<td>Flunitrazepam-d7</td>
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<tr>
<td>Buspiron</td>
<td>Anxiolytic</td>
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<td>5.0 / 10</td>
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<tr>
<td>Carbamazepine</td>
<td>Anticonvulsant, mood stabilizer</td>
<td>Carbamazepin-d10</td>
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<td>13 / 30</td>
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<tr>
<td>Carbamazepine epoxide</td>
<td>Carbamazepine metabolite</td>
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<td>Quantitative</td>
<td>50–5000</td>
<td>9.0 / 26</td>
</tr>
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<td>Citalopram</td>
<td>Antidepressant SSNRI</td>
<td>Citalopram-d6</td>
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</tr>
<tr>
<td>Clozapine</td>
<td>Benzodiazepine</td>
<td>Temazepam-d5</td>
<td>Quantitative</td>
<td>25–500</td>
<td>5.8 / 17.6</td>
</tr>
<tr>
<td>Clomethiazole</td>
<td>Sedative, hypnotic, anticonvulsant</td>
<td>-</td>
<td>Qualitative</td>
<td>-</td>
<td>5.0 / -</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>Benzodiazepine</td>
<td>Clonazepam-d4</td>
<td>Quantitative</td>
<td>2.5–100</td>
<td>1.3 / 2.5</td>
</tr>
<tr>
<td>Clonazepam, 7-amino-</td>
<td>Benzodiazepine metabolite</td>
<td>7-Aminoflunitrazepam-d7</td>
<td>Quantitative</td>
<td>2.5–100</td>
<td>0.44 / 1.6</td>
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<td>5–500</td>
<td>1.1 / 3.5</td>
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<td>Clozapine, nor-</td>
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<td>0.85 / 2.6</td>
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<td>Benzodiazepine</td>
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<tr>
<td>Diazepam, nor-</td>
<td>Benzodiazepine / benzodiazepine metabolite</td>
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<td>2.4 / 4.5</td>
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<td>Diphenhydramine</td>
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</tr>
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<td>Doxepin</td>
<td>TCA</td>
<td>Doxepin-d3</td>
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</tr>
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<td>Doxepin, desmethyl-</td>
<td>TCA metabolite</td>
<td>Nortriptylin-d3</td>
<td>Quantitative</td>
<td>5–500</td>
<td>1.1 / 2.3</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>Benzodiazepine</td>
<td>Flunitrazepam-d7</td>
<td>Quantitative</td>
<td>2.5–100</td>
<td>0.39 / 1.4</td>
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<td>Flunitrazepam, desmethyl-</td>
<td>Benzodiazepine</td>
<td>Flunitrazepam-d7</td>
<td>Quantitative</td>
<td>1–100</td>
<td>0.66 / 0.7</td>
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<tr>
<td>Flunitrazepam, 7-amino-</td>
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<td>7-Aminoflunitrazepam-d7</td>
<td>Quantitative</td>
<td>2.5–100</td>
<td>0.68 / 2.0</td>
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<tr>
<td>Fluoxetine</td>
<td>Antidepressant SSNRI</td>
<td>Fluoxetine-d6</td>
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<td>1.6 / 2.5</td>
</tr>
<tr>
<td>Fluoxetine, nor-</td>
<td>Antidepressant SSNRI metabolite</td>
<td>-</td>
<td>Qualitative</td>
<td>-</td>
<td>2.2 / -</td>
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</tbody>
</table>
### Table 1: 65 target analytes including ISTD, validation status, calibration range, and LOD/LOQ for each analyte

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</thead>
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<td>Flupirtine</td>
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<td>Risperidon-d4</td>
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<td>50–5000</td>
<td>11 / 29</td>
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<tr>
<td>Flurazepam</td>
<td>Benzdiazepine</td>
<td>-</td>
<td>Qualitative</td>
<td>-</td>
<td>5.0 / -</td>
</tr>
<tr>
<td>Flurazepam, desalkyl-</td>
<td>Benzdiazepine metabolite</td>
<td>Temazepam-d5</td>
<td>Quantitative</td>
<td>5–500</td>
<td>1.8 / 9.6</td>
</tr>
<tr>
<td>Fluvoxamin</td>
<td>Antidepressant SSNRI</td>
<td>Fluoxetin-d6</td>
<td>Quantitative</td>
<td>5–500</td>
<td>0.94 / 3.9</td>
</tr>
<tr>
<td>Lorazepam</td>
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<tr>
<td>Lorometazepam</td>
<td>Benzdiazepine</td>
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<td>Quantitative</td>
<td>2.5–100</td>
<td>0.96 / 1.6</td>
</tr>
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<td>5.1 / 23</td>
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<tr>
<td>Methadone</td>
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<td>Methadon-d9</td>
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<td>6.8 / 21</td>
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<td>Methadone metabolite</td>
<td>EDDP-d9</td>
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<td>6.3 / 18</td>
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<td>Midazolam</td>
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<td>Mirtazapin</td>
<td>TCA</td>
<td>Clozapin-d4</td>
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<td>1.3 / 3.5</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>Benzdiazepine</td>
<td>Flunitrazepam-d7</td>
<td>Quantitative</td>
<td>10–500</td>
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<tr>
<td>Nitrazepam, 7-amino-</td>
<td>Benzdiazepine metabolite</td>
<td>-</td>
<td>Qualitative</td>
<td>-</td>
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<td>Nortriptylan</td>
<td>TCA, metabolite amitriptylin</td>
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<td>1.2 / 3.1</td>
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<td>Opiopramol</td>
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<tr>
<td>Oxazepam</td>
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<td>1.0 / 3.0</td>
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<td>Perphenazine</td>
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<td>-</td>
<td>2.0</td>
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<td>5–500</td>
<td>2.5 / 5.0</td>
</tr>
<tr>
<td>Quetiapin</td>
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<td>Doxepin-d3</td>
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<td>5–500</td>
<td>1.1 / 4.0</td>
</tr>
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<td>Risperidone</td>
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<td>Risperidon-d4</td>
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<td>1.6 / 3.8</td>
</tr>
<tr>
<td>Paliperidone (Risperidone, 9-hydroxy-)</td>
<td>Antipsychotic / anti-psychothic metabolite</td>
<td>Doxepin-d3</td>
<td>Quantitative</td>
<td>5–500</td>
<td>1.1 / 3.9</td>
</tr>
<tr>
<td>Sertralin</td>
<td>Antidepressant SSNRI</td>
<td>Sertralin-d3</td>
<td>Quantitative</td>
<td>10–500</td>
<td>1.9 / 6.2</td>
</tr>
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<td>Temazepam</td>
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<td>Temazepam-d5</td>
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<tr>
<td>Tetrazepam</td>
<td>Benzdiazepine</td>
<td>Diazepam-d5</td>
<td>Quantitative</td>
<td>10–500</td>
<td>4.0 / 6.2</td>
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</table>
The comprehensively automated SPE–LC–MS/MS method described in this article was developed at the Institute of Legal Medicine in Cologne, Germany, based on a manual SPE workflow from the Institute of Legal Medicine in Muenster, Germany.

**Experimental**

For automation of the sample preparation steps and injection of the final extract into the LC–MS/MS system a MultiPurpose Sampler (MPS, Gerstel) was employed. The automated system consisted of a Dual Head MPS (Gerstel) with a 2.5-mL syringe on the left head for all sample preparation steps and a 100-μL syringe for injection of the prepared sample extract into the LC–MS/MS (1200 SL LC and 6460 MS, Agilent Technologies). Modules for SPE, extract evaporation, and solvent reservoirs were adapted to the autosampler.

Initially a 250 μL serum or plasma sample was added and mixed with 25 μL of an internal standard (ISTD) solution in a 2-mL vial. In the case of a hemolytic or post-mortem specimen a protein precipitation step with acetonitrile was performed before the SPE. Sample vials were then placed in the autosampler and all further preparation steps, including injection to the analysis system, were executed automatically. Since all the included compounds can be protonated at acidic pH values, a cation exchange sorbent was chosen for analyte enrichment and cleanup (Chromabond HR-XC, 60 mg, 45 μm, 3 mL format, REF 730956P45MPS, Macherey-Nagel). The SPE automation relies on standard SPE cartridges that are cut and equipped with a transport adapter at the top and a disposable canula on the Luer port at the bottom. The cartridge was conditioned by injecting each 1 mL of methanol, water, and phosphate buffer pH 6. The sample was diluted with 1 mL of phosphate buffer pH 6, loaded onto the SPE cartridge, and analytes were bound by the nonpolar backbone of the sorbent material followed by washing steps with 1 mL phosphate buffer and 70:30 (v/v) water–methanol. During these steps, medium-polar and polar interferences were washed away. In the next step the cartridge was washed with 0.1 M HCl protonating the analytes and binding them to the cation exchange groups of the sorbent. A sequence of washing steps, including methanol, followed in order to further remove interfering compounds bound to the nonpolar backbone of the sorbent while the analytes were bound to the cation exchange groups. In between these steps and directly before analyte elution, the cartridge was dried in a stream of nitrogen delivered through the 2.5-mL syringe, which was connected to a gas supply. Compounds of interest

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**Table 1:** 65 target analytes including ISTD, validation status, calibration range, and LOD/LOQ for each analyte

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<th>Drug Class</th>
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<th>Calibration Range (ng/mL)</th>
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<td>Tetrazepam, nor-</td>
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<td>Diazepam-d5</td>
<td>Semi-quantitative</td>
<td>10–100</td>
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<td>Triazolam</td>
<td>Benzodiazepine</td>
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<td>Quantitative</td>
<td>1–100</td>
<td>0.58 / 0.8</td>
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<td>Triazolam, 1-hydroxy-</td>
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<td>Trimipramin</td>
<td>TCA</td>
<td>Trimipramin-d3</td>
<td>Quantitative</td>
<td>5–500</td>
<td>1.2 / 4.0</td>
</tr>
<tr>
<td>Venlafaxin</td>
<td>Antidepressant SSNRI</td>
<td>Venlafaxin-d6</td>
<td>Quantitative</td>
<td>5–500</td>
<td>0.93 / 2.3</td>
</tr>
<tr>
<td>Venlafaxin, O-desmethy-</td>
<td>SSNRI metabolite</td>
<td>Doxepin-d3</td>
<td>Quantitative</td>
<td>5–500</td>
<td>1.2 / 1.9</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>Sedative, hypnotic (Z-drug)</td>
<td>Clonazepam-d4</td>
<td>Quantitative</td>
<td>2.5–100</td>
<td>0.60 / 1.9</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>Sedative, hypnotic (Z-drug)</td>
<td>Zolpidem-d6</td>
<td>Quantitative</td>
<td>10–500</td>
<td>2.1 / 7.9</td>
</tr>
<tr>
<td>Zopiclone</td>
<td>Sedative, hypnotic (Z-drug)</td>
<td>Zolpidem-d6</td>
<td>Quantitative</td>
<td>2.5–100</td>
<td>0.62 / 1.7</td>
</tr>
<tr>
<td>Z-Amino-5-chloropyridine (ACP)</td>
<td>Decomposition product of zopiclone</td>
<td>-</td>
<td>Qualitative</td>
<td>-</td>
<td>5.0 / -</td>
</tr>
</tbody>
</table>
were eluted two times with 500 μL of a mixture of ethyl acetate and ammonia. The eluates were collected in vials fitted with septum caps and were subsequently evaporated to dryness in the evaporation module for 10.5 min at 50 °C under shaking and reduced pressure comparable to a rotary evaporator. The extracts were reconstituted in 100 μL of a 85:15 (v/v) water–acetonitrile mixture containing 0.1% formic acid. A 30 μL aliquot of this solution was injected into a 5 μL sample loop for transfer onto the LC column, a 150 × 2 mm, 2.7 μm Nucleoshell RP 18 (Macherey-Nagel). Analytes were separated during a 15-min gradient run with a flow of 0.3 mL/min employing water and acetonitrile, both including 0.1% formic acid, and detected in dynamic multiple reaction monitoring mode (MRM). For each target compound two MRM transitions were chosen, one quantifier and one qualifier, in addition to one MRM transition for deuterated internal standards (ISTD) used for quantification.

Results and Discussion
The analysis method was completely validated according to guidelines of the GTFCh (8) for 52 of the 65 target analytes (Table 1). Seven further compounds were considered as “semi-quantitative” because the complete validation data had not yet been collected. For an additional six compounds a “qualitative” detection can be performed by having established a cut-off value, which includes evaluation of extraction recovery and matrix effects. With regard to “semi-quantitative” and “qualitative” compounds, quantitation is not required on a regular basis at the laboratory in Cologne.

Validation comprised establishing limits of quantification/detection (LOQ/LOD) and the linear range. Furthermore, precision (repeatability and time-different intermediate precision) and accuracy were tested at low and high concentrations inside the linear range as well as the extraction recovery and matrix effects. Limits of quantification were between 1–35 ng/mL, and were below 10 ng/mL for the vast majority of compounds. The linear ranges reached upper values between 100–2500 ng/mL. For practical reasons, in many cases the lowest calibration point was chosen slightly above the LOQ to facilitate the preparation of calibration solutions. Precision data expressed as relative standard deviation were mainly below 8% and often even below 5%, proving the excellent repeatability of such an automated analysis method. Spiked blank matrix was used for calibration, blank matrix

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spiked with internal standard only as matrix blank sample, and external quality control samples were analyzed in every sequence. In addition, the laboratory successfully took part in available “round robin” tests for the following compound classes: benzodiazepines, opioids, z-drugs, tricyclic antidepressants, and selective serotonin noradrenaline reuptake inhibitors. The developed method is included in the DIN EN ISO/IEC 17025:2018 accreditation of the forensic toxicology laboratory at the Institute of Legal Medicine in Cologne. It is employed routinely for serum, plasma, whole blood, and post-mortem specimens, adding up to roughly 1500 samples analyzed so far. Since a couple of compounds cannot be detected by immunochemical pre-testing, the described SPE–LC–MS/MS method is used as a multi-targeted screening that includes confirmation analysis and quantitation, if necessary. Before implementation of this method the parameters were contained in two separate workflows running on a standalone SPE extractor. Each of them in two separate workflows running on a standalone SPE extractor. Each of them needed 0.5 mL of sample resulting in a total required volume of 1 mL, while today only 0.25 mL is needed for the complete set of analytes. The former methods demanded supervision and several manual interventions, such as evaporation of the extracts and reconstitution, which required the undivided attention of laboratory personnel, making it difficult to perform other tasks. Comprehensive automation of the workflow, including LC injection, reduces the workload and gives laboratory personnel time for other important tasks, such as data review or developing new analysis methods. Due to the extensive cleanup and enrichment procedure, good quality chromatograms with very low levels of interfering compounds are achieved (Figure 1) and the maintenance effort for the LC–MS/MS instrument is reduced. Analyte peaks can be identified unambiguously and automated integration results in accurate quantification without the need for reintegration for the vast majority of peaks.

Conclusions
This comprehensive automation of complex sample preparation workflows, including the injection into an analysis system, greatly reduces the manual workload. Data quality is high without depending on manual intervention and reintegration. In addition to the application described in this article, the laboratory in Cologne also employs a similar setup coupled to a GC–MS/MS system for the automated determination of cannabinoids and metabolites in serum and hair. These examples illustrate the benefits of implementing automated sample preparation methods into laboratory workflows.

Acknowledgements
The author would like to thank Clara Reinartz, Tobias Kieliba, Maren Fussberger, and Hilke Andrensen-Streichert Ph.D. from the Institute of Legal Medicine in Cologne, Germany, for carrying out the major part of method development, the validation, and for sharing all relevant information. Jennifer Schuurenkamp Ph.D. from the Institute of Legal Medicine in Muenster, Germany, is acknowledged for the development of the initial manual analysis method.

References

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Octagon Therapeutics Receives Agilent Golden Ticket
Agilent Technologies Inc. (California, USA) has announced that Octagon Therapeutics (Massachusetts, USA) have been awarded an Agilent Golden Ticket at LabCentral. The Golden Ticket provides funding for one scientist’s laboratory bench space for a year, including the benefit of LabCentral’s shared infrastructure and services.

Octagon’s mission is to discover and develop better treatments for autoimmune disease. The immune cells that drive autoimmune disease are genetically identical to healthy immune cells, but have become pathologically activated in specific ways. Octagon’s platform enables the identification of the hallmarks of immune dysregulation, allowing the precise treatment of autoimmune conditions without damaging healthy immune functioning. The ability to treat an autoimmune disease’s underlying drivers without suppressing the immune system is necessary to prevent secondary complications.

“We are thrilled to have won the Agilent Golden Ticket,” said Isaac Stoner, president and chief executive officer of Octagon Therapeutics. “Octagon has identified important new biology related to the underlying causes of autoimmune disease. The analytical chemistry expertise and instrumentation that Agilent has provided to LabCentral will be critically important as we turn these insights into new medicines, and the vibrant LabCentral community will help to shape our company culture as we grow.”

“It’s great to see Octagon awarded with Agilent’s 2021 Golden Ticket. We are excited about the potential of their autoimmune-therapeutics platform,” said Johannes Fruehauf, co-founder and president of LabCentral.
For more information, please visit: www.agilent.com

LCGC Europe and HTC-17 Launch 2022 HTC Innovation Award

LCGC Europe are now accepting nominations for the 2022 HTC Innovation Award through to 30 June 2021.

“It is such a privilege to showcase the work of the brightest scientists around the globe who are making pioneering contributions to the field of separation sciences,” said Michael Hennessy Jr., president and CEO of MJH Life Sciences, parent company of LCGC Europe. “We are delighted to honour and recognize the achievements of a talented scientist who is continuing to propel the specialty forward, making great strides in their career and research.”

The 2022 HTC Innovation Award winner will be selected by the HTC-17 Scientific Committee and the HTC-17 Industry Board, based on the following criteria:

• The winner has made a pioneering contribution to the field of separation sciences by introducing new methodologies, new instrumentation, or new techniques in the field, with a strong focus on applicability.
• Applications are open to scientists who have under 15 years of experience after completing their PhD.
• Applications from separation scientists worldwide are welcomed. LCGC Europe readers can nominate themselves or others.

Previous winners have included: Ryan Kelly of Brigham Young University, Utah, USA, who was nominated and awarded the honour based on his outstanding contributions to the field of microcolumn separations involving hyphenation, and Carolin Huhn of Eberhard Karls Universität Tübingen, Germany, who received the inaugural award for her work on a modular instrumental approach where the modular building blocks can be flexibly combined in relation to particular analytical tasks to implement a broad range of different elements of chemical analysis.

The 2022 HTC Innovation Award recipient will be presented with a plaque honouring their accomplishment at the HTC-17 conference, which will be held in Ghent, Belgium, at Conference Center Het Pand, the culture and congress center of Ghent University, from the 26–28 January 2022.

For more information and to submit a nomination, click here: https://bit.ly/3wcb3dW
Shimadzu Receives Red Dot Award

Shimadzu (Duisburg, Germany) has been awarded a “Red Dot: Best of the Best Award 2021” in the product design category for its MALDImini-1. The prize is the highest award in this competition and is reserved for the best products in a category of groundbreaking design.

“The winners of the ‘Red Dot: Best of the Best’ have demonstrated unique design competency. Their objects set trends in product design and give a glimpse of future developments,” said Professor Dr. Peter Zec, founder and CEO of Red Dot.

With a history of more than 60 years, the Red Dot Design Award appraises the best products created every year. Alongside the German iF Award and the US IDEA Award, it is one of the major three design awards in the world.

Shimadzu’s MALDImini-1 digital ion trap mass spectrometer fits on a space the size of a DIN A3 sheet, allowing installation in places where mass spectrometers could not previously fit. It enables ion trapping up to 70,000 Da and the MS/MS and MS3 functionality of the digital ion trap allows researchers to perform comprehensive structural analyses.

For more information, please visit: www.shimadzu.eu/awards-of-shimadzu

Differentiating Frontotemporal Dementia Through Metabolomics

Researchers have investigated the plasma metabolite profile of patients with frontotemporal dementia (FTD) and Alzheimer’s disease (AD) using an untargeted metabolomic approach in combination with gas chromatography–mass spectrometry (GC–MS) (1).

Frontotemporal dementia (FTD) is a neurodegenerative disorder characterized by progressive impairment in behaviour, executive function, and language. It is one of the most common causes of neurocognitive disorder in people under 65 years old with around 10 in every 100,000 persons likely to be afflicted. The most common form of FTD is a behavioural variant (bvFTD), which is clinically different to atypical AD cases and which it can sometimes be mistaken for. Generally, survival is shorter and cognitive decline is faster than with typical AD cases. Therefore, a differential diagnosis between bvFTD and atypical AD cases is crucial.

Currently, to differentiate between bvFTD and AD cases requires an evaluation of cognition and cerebrospinal fluid (CSF) biomarkers as well as a neuroimaging investigation and genetic mutations screening; however, an accurate diagnosis still poses a clinical challenge in specific cases. Furthermore, the collection of CSF requires an invasive lumbar puncture procedure, access to molecular neuroimaging methods can be restricted, and no blood biomarkers have been identified.

An alternative to diagnosis through these methods could come with the investigation of metabolites by Tsuruoka et al. (2), who identified six serum metabolites that were significantly increased in patients with dementia as well as 45 additional metabolites identified as candidate markers that could discriminate patients with dementia from cognitively healthy controls. As such, researchers aimed to investigate the plasma metabolite profile of patients with bvFTD compared to AD patients and cognitively healthy individuals using an untargeted metabolomic approach, with hopes to identify biological mechanisms and possible biomarkers for bvFTD diagnosis.

Results found a reduction in the levels of palmitoleic, oleic, and lauric acids in the bvFTD group compared to the AD group, however, no significance after multiple comparison correction was observed. Reduced levels of creatinine, glycine, tryptophan, uric acid, hypoxanthine, serine, valine, threonine, isoleucine, homoserine, methionine, glutamic acid, capric acid, tartaric acid, fumaric acid, and myo-inositol, metabolites related to glycine/serine/threonine, alanine/aspartate/glutamate pathways and aminoacyl-tRNA biosynthesis, were also found in the bvFTD group when compared to controls. This led researchers to theorize that bvFTD patients may present an impairment of amino acid metabolism and the translation process.

Researchers hope this study can encourage further and more robust studies to fully explore the possibility of biomarkers for the diagnosis of this dementia form.—L.B.

References

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News In Brief

• The LCGC Blog: Celebrating HPLC Pioneer Elmar Piel—In this LCGC Blog, James Grinias invites Ron Majors to join him in writing about scientist, Elmar Piel. Perhaps unfamiliar to many chromatographers, Piel’s pioneering work on early high performance liquid chromatography (HPLC) was pivotal to the development of modern-day HPLC. Read Here>>

• Surfing on Mobile Phase, Part 2: Impact of Mobile Phase Composition Waves on Retention in LC—The most commonly used designs for modern liquid chromatography (LC) pumps produce mobile-phase streams with small short-term variations in mobile-phase composition. Understanding the origin of these variations, and their effects on chromatographic performance, can help us develop more robust methods, and monitoring for this aspect of pump performance is an important step in using the best troubleshooting tool—preventative maintenance. Read Here>>

• Rising Stars of Separation Science: Mariosimone Zoccali—In the first in our series of interviews with early career researchers we spoke to Mariosimone Zoccali, assistant professor of analytical chemistry at the University of Messina, Italy, about his innovative work to develop a powerful analytical method using online SFE–SFC–QQQ–MS to analyze important bioactive molecules. Read Here>>

• Flying High with Sensitivity and Selectivity: GC–MS to GC–MS/MS—Mass spectrometry (MS) is the most powerful detector available for gas chromatography (GC). This article reviews the fundamentals of MS/MS and how they relate to MS as a detector for GC, then examines scenarios where use of GC–MS/MS can solve complex problems. Read Here>>

• Tips & Tricks GPC/SEC: Separation Range and Resolution—This instalment describes the interplay between column length, pore size distribution, and particle size to optimize GPC/SEC separations. Read Here>>

• Determination of Microplastic Mass Content by Thermal Extraction Desorption Gas Chromatography–Mass Spectrometry—A novel analysis technique has been developed for the determination of microplastics (MPs) in complex environmental samples using thermal extraction desorption gas chromatography–mass spectrometry (TED-GC–MS). Read Here>>

PerkinElmer Acquires ES Industries
PerkinElmer, Inc. (Massachusetts, USA) has announced the acquisition of ES Industries (New Jersey, USA), adding their columns and chemistries to its LC consumables portfolio. ES Industries is known for HPLC and SFC column chemistries. The team has over 40 years of experience delivering columns with reliability, scalability, and reproducibility that are used routinely for method development processes, LC–MS analysis, quality control, and preparative purification. For more information, please visit: https://www.perkinelmer.com/category/liquid-chromatography-hplc-uhplc-instruments; https://esind.com/

Sartorius Opens New Facility
Sartorius will open a new 58,000 square-foot state-of-the-art manufacturing facility in Havant, Hampshire, UK. The opening marks the official relocation of more than 90 employees to the new facility, located just a few miles away from the former site in Portsmouth. In addition to a 28,000 square-foot production area, the centre accommodates a 4000 square-foot customer test laboratory to demonstrate the functionality of Sartorius equipment. “The new facility in Havant will be an important part of our global operations network as it will enable our business to grow to meet the requirements of the expanding industry,” said Thorsten Peuker, Head of Operations, BPS Systems at Sartorius. For more information, please visit: www.sartorius.com
Driving Under the Influence of Drugs: PESI for the Measurement of Illicit Drugs in Saliva

Pauline Griffeuille1, Sylvain Dulaurent1, Stephane Moreau2, and Franck Saint-Marcoux1,
1Unit of Biological and Forensic Toxicology, Department of Pharmacology and Toxicology,
Limoges University Hospital, France, 2Shimadzu Europe, Duisburg, Germany

This article demonstrates how a new probe electrospray ionization tandem mass spectrometry (PESI-MS/MS) method can be used to measure illicit drugs in saliva, confirming positive tests for driving under the influence of drugs (DUID) in France. A new method was developed for the measurement of illicit drugs in saliva using a PESI-MS/MS approach. Satisfactory results were obtained for eight opiates, amphetamines, and cocaine derivatives.

Probe electrospray ionization (PESI) is an ambient ionization method where a disposable solid needle is used as a sample probe, and electrospray ionization (ESI) is used as an emitter. Coupled with a mass spectrometer, it allows very fast analyses with minimal sample preparation. Unlike PESI, traditional mass spectrometry (MS) ion sources have to be maintained in a vacuum, and sample preparation is more extensive.

In France, confirmation of a positive test for driving under the influence of drugs (DUID) is performed in an authorized laboratory using a liquid chromatography (LC)–MS/MS method. How can a new method be developed for the measurement of illicit drugs in saliva using a PESI-MS/MS approach?
The following conditions were defined: 50 μL of saliva was mixed in a methanol–ethanol–ammonium formate buffer. After
adding deuterated internal standards, a 10 μL measure was set in the PESI system. A scheduled multiple reaction monitoring (MRM) approach with two transitions per compound was developed. The total run time was about 1.5 min. Based on ISO 15189, validation steps included the determination of the limit of detection (LOD) and the limit of quantification (LOQ), as well as repeatability and reproducibility analysis.

Satisfactory results were obtained for eight opiates, amphetamines, and cocaine derivatives. LODs in particular were below the limits defined by the law (10 ng/mL). In a sample of 52 real cases, the new method yielded results comparable to those of the LC–MS/MS method routinely used in the laboratory.

It is feasible to use PESI-MS/MS for a very fast measurement of illicit drugs in saliva. Further analytical improvements are needed to integrate all the compounds required by law.

**Illicit Drug Analysis for Drivers**

Usually, when a driver is suspected of consumption of illicit drugs, roadside testing is performed with an immunological kit based on an oral fluid (OF) or a urine sample. If positive, this test has to be confirmed in a second sample by a gold standard method, such as LC–MS/MS.

The legislation of most countries concerning DUID is based on impairment ("effect of..."
the drug”) or on analytical limits (“cut-off” approach) (1). In some countries, including France, this confirmation is now typically (almost systematically) performed using an OF sample, and the regulatory approach does not apply a per se law (such as the driver is sentenced if the drug level is above a predefined limit), but based on a zero tolerance law (that is, driving with a measurable amount constitutes an offense). Consequently, laboratories have to detect a list of illicit drugs at a minimum concentration.

In France, for example, this “cut-off” is 10 ng/mL for cocaine, benzoylecgonine (BZE), morphine, 6-monoacetylmorphine (6-AM), amphetamine, methamphetamine, 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxy-N-methylamphetamine (MDMA) (2).

What is PESI?

PESI is an ambient ionization method. It was developed by Kenzo Hiraoka of the University of Yamanashi, Japan, who published the first article on the subject in 2007 (3). Briefly, it contains a disposable solid needle used as a sample probe and an
Electrospray ionization (ESI) that is used as an emitter (3). This probe needle repeatedly moves down (that is, “dives” into the sample) and then moves up when ionization occurs. The compounds are ionized periodically and their resultant ions are pushed into the MS/MS system. Consequently, when coupled with a MS system, the PESI makes a direct analysis of liquid samples and solid samples possible without pre-treatment.

**Publications and Applications of the PESI Technique**

Different applications of the PESI technique have been reported in clinical toxicology, forensic science, and metabolic profiling. Zaitsu et al. applied PESI to measure 26 endogenous metabolites of mice liver, including amino acids, organic acids, and sugars (4). The same team also applied PESI to monitor eight cerebrum metabolites related to central energy metabolism in an isoflurane-anesthetized mouse (5).

Johno et al. proposed an automatic diagnosis system of atherosclerotic disorders by measuring metabolites including cholesterol sulphate and a phospholipid, both promising new biomarkers of atherosclerosis (6). Usui et al. applied the PESI technique with LC and triple quadrupole (QQQ)-MS/MS for the detection of acetaminophen in less than 2 min (7). The same team also proposed a similar approach for the diagnosis of acute intoxications to glyphosate (8).

**A PESI-MS/MS Method for Illicit Drugs**

In this study, a PESI-MS/MS application for the determination of some illicit drugs in OF was developed. From a practical point of view, the main objective was to obtain performances in accordance with French legislation as described previously. The principle of the method can be summarized as follows:

50 μL OF samples were diluted in 450 μL of a 25:25:50 methanol–ethanol–ammonium formate 10 mM buffer. In a previous paper, Usui et al. (7,8) proposed an ethanol–ammonium formate buffer, but in preliminary studies it was observed that either methanol or ethanol could be necessary depending on the molecule.

From this 500 μL, 180 μL was mixed with 20 μL of a solution containing eight deuterated internal standards at 50 ng/mL. Finally, a 10 μL sample of diluted OF was placed on the dedicated plastic sample plate and set in the PESI ion-source.

LC–QQQ-MS/MS with a PESI ion source was used for the determination of cocaine, BZE, morphine, 6-MAM, methamphetamine, MDEA, MDA, and MDMA. A scheduled MRM approach with simultaneous detection of two transitions of two compounds, acquired in the positive mode, was developed (Figure 1). For an MRM approach, three transitions for each compound are usually recommended. However, by increasing the analysis time, desolvation of the solvents was observed.

The total run time was 1.31 min for detection of the eight compounds. The whole procedure was validated according to the EMEA guidelines (9). A summary of performances is given in Table 1.

Along the validation process, hundreds of OF samples free of illicit drugs were analyzed. Particular matrix effects for the eight molecules could not be observed. A typical extracted ion count of morphine, obtained for an OF sample spiked at 10 ng/mL, is presented in Figure 2.

The overall procedure was applied to 52 real cases of DUID that were also analyzed by a validated LC–MS/MS method. Briefly, the method is routinely used by the laboratory of Limoges University, France (accredited
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Table 1: Summary of performances

<table>
<thead>
<tr>
<th>Molecule</th>
<th>LOD of the method (ng/mL)</th>
<th>LOD expected by the law (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZE</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>6-AM</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>MDEA</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td>0.5</td>
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</tr>
</tbody>
</table>

According to ISO 15189 and uses a QuEChERS sample preparation and LC–QQQ–MS/MS method (10).

Seventy-three molecules were detected in these 52 samples: MDMA (n = 17), MDA (n = 1), cocaine (n = 26), BZE (n = 22), 6-MAM (n = 4), and morphine (n = 3). Each compound was systematically detected with both methods and excellent correlations were calculated between the new PESI-MS/MS method and the classical LC–MS/MS method. This study is illustrated in Figure 3, which reports the regression analysis for cocaine, BZE, and MDMA.

Conclusion

These experiments demonstrate the feasibility of using a PESI-MS/MS approach for the measurement of illicit drugs in saliva. With this approach, the detection is performed in about 1.5 min without an extraction procedure. The current procedure includes only eight molecules, and further studies are needed to increase the number of illicit drugs that could be tested in a single run.

References


Pauline Griffeuille is an intern in pharmacy. In November 2020, she started a PhD thesis in analytical chemistry. The main objective of her work is to develop analytical tools based on a PESI-MS/MS approach for applications in forensic, clinical, and occupational toxicology. This thesis is supervised by Sylvain Dulaurent and Franck Saint-Marcoux.

Sylvain Dulaurent is an engineer in analytical chemistry and obtained a Ph.D. in 2010. He is currently the analytical scientist responsible for the Clinical and Forensic Toxicology Unit of the Limoges University Hospital (France). He has developed and published numerous analytical solutions for the determination of pesticides residues, drugs, and illicit drugs. He is also a legal expert in forensic toxicology with daily activity dedicated to DUID.

Stephane Moreau obtained his diploma from INSA in fine chemistry and engineering with a specialization in chemical process engineering in 1994. He then started his professional career in laboratory equipment distribution before joining the brand new Shimadzu France subsidiary in 2002. Since then, Moreau has held various positions to develop the MS range of the business. Since September 2013, he has been product manager for the MS range with Shimadzu Europe. Franck Saint-Marcoux works equally for the university and the hospital, where he is in charge of clinical toxicology, environmental toxicology, and forensic science for both. He is responsible for the Clinical and Forensic Toxicology Unit of the Limoges University Hospital (France). He is full professor of toxicology at the University Hospital of Limoges. He has received different scientific awards from international societies, and is author or co-author of over 80 papers in peer-reviewed international journals.

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Novel GC–MS Software Programs for Analyzing Seized Drug Samples

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There has been a proliferation of novel psychoactive substances (NPS) in drug seizures. These drugs are synthesized with minor modification to the chemical structure of existing drugs. Specifically, many fentanyl derivatives have shown up in drug seizures including isomeric variations that produce nearly identical mass spectra. These compounds are generally present at lower concentrations making identification difficult. Gas chromatography–mass spectrometry (GC–MS) is the workhorse in forensic chemistry for identification of controlled substances. Using two software programs—retention time locking (RTL), and deconvolution reporting software (DRS)—the authors developed an analysis capable of identifying and distinguishing isomeric forms. An example distinguishing para-methylacetylfentanyl and ortho-methylacetylfentanyl is described.

Forensic chemists have been encountering new materials in their drug casework (1,2). These novel psychoactive substances (NPS) are generally variations of a known controlled substance where the core structure is intact, but modifications have been made to various positions of the core structure. The core structure creates structural and positional isomers and analogous drugs that retain the pharmacological effects on the body, but may have different analytical properties in terms of retention time and mass spectral appearance (3).

A significant finding seen in drug seizures is fentanyl. Fentanyl is many times more powerful
than other opiates such as morphine or heroin. It is added to illicit drugs due to the powerful effects that can be experienced from small doses. As an unintended consequence, many more people are dying from the use of drugs laced with fentanyl. In addition to fentanyl, numerous fentanyl derivatives have been identified in drug seizures, including isomeric variations that produce nearly identical mass spectra.

The rise in the number of fentanyl derivatives, coupled with the fact that they can be present in samples at low concentrations, has made data processing a more complex process for identification and confirmation.

Preliminary testing generally consists of non-mass spectral testing such as colour tests, which can give an indication as to the identity. As seized drug mixtures have become more complex, these preliminary tests provide much less in terms of drug identity, especially for instances where the active component, that is fentanyl or fentanyl derivatives, is present in relatively small amounts.

**NMS Labs and Process Description**

NMS labs is a forensic chemistry and forensic toxicology laboratory that has performed forensic testing for over 50 years. Their testing procedures are designed to meet the strict requirements of civil and criminal justice professions. NMS uses state-of-the-art instrumentation to provide results backed by NMS lab’s accreditation and expertise. The

**Figure 1:** One month of daily retention times are shown for three instruments using RTL (teal) and without RTL (blue).
forensic chemistry division is a series of drug testing laboratories throughout the United States involved with testing at the local, state-wide, and national level. Drug testing involves the recommended flow of testing recommended by SWGDRUG to identify seized drugs, starting with standard preliminary test procedures for initial identification. This includes various colour tests, pharmaceutical identification (where applicable), and, in certain cases, microscopic analysis. Confirmatory testing is generally performed by gas chromatography coupled to a mass spectrometer (GC–MS). The laboratories have additional testing capability such as liquid chromatography (LC)–MS/MS, Fourier-transform infrared spectrometry (FT-IR), and high performance liquid chromatography (HPLC)–UV. Additional identifying tests are run by the Center for Forensic Science Education and Research for identification of emerging drugs of interest. Testing is performed using LC–quadrupole time-of-flight (QTOF) mass spectrometry for more in-depth molecular identification.

GC–MS is the workhorse in forensic chemistry for the identification of controlled substances. This analysis platform has found favour due to three primary considerations: instrument cost, ease of use, and utility of multiple GC–MS databases from multiple sources. Electron ionization produces nearly identical mass spectra on multiple systems from multiple vendors. Most drug testing laboratories have adopted GC–MS for these reasons. Additionally, GC–MS is deemed a top tier assay for confirmation of drug identity. In the United States, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommends that drug identification be performed using at least one test that provides structural information (designated a category A technique), which includes mass spectrometry (4).

As previously described, NMS Labs performs forensic chemistry testing and maintains a network of testing laboratories in the United States across multiple states. It is important that standardized analytical testing is provided at all sites. These sites analyze many thousands of samples annually, and it is necessary to have confidence that all samples are correctly analyzed and reported independently of where the sample was received. Standardized data acquisition and processing also allows inter-laboratory technical review of casework.

Figure 2: Deconvolution is achieved by grouping together ions whose EICs have the same shape and apex retention time to create a spectrum free of interferences from overlapped peaks.

Figure 3: Deconvolution data indicating the ability to distinguish these three isomers of methylethcathinone. Including the retention time improves the match factor for 3-methylethcathinone and 4-methylethcathinone.
The Analytical Approach to Testing

NMS Labs has established procedures for extraction and subsequent GC–MS analysis for seized drug evidence. The data processing procedure incorporates multiple software programs, retention time locking (RTL), deconvolution reporting software (DRS), and AMDIS (Automated Mass Spectral Deconvolution and Identification System) (5,6). Using these programs, the laboratory developed an analytical method capable of identifying and distinguishing many isomeric forms of evolving drugs.

RTL software allows for retention time reproducibility despite changes in column dimensions through variation in production and maintenance associated with normal use in the laboratory. Retention time locking calibration calculates how changes in pressure affect retention time. Using this calibration, slight alterations are made to the carrier gas flow by adjusting the pressure in the method and from there to the retention times of analytes. This can be used to accommodate column clipping and even installation of a new column. The RTL program will re-calculate the pressure necessary to provide the desired retention time after each maintenance event. The laboratory can therefore maintain a database of drugs with the inclusion of retention time in the database entry. This method can subsequently be propagated to other instruments in the laboratory and to instruments throughout the network. Testing was performed on other compounds to demonstrate the same level of repeatability.

The second software application is the use of DRS and AMDIS. AMDIS provides a comprehensive means of searching data files for target compounds through an examination of extracted ion profiles to determine the presence of individual compounds. Deconvolution is achieved when masses exhibiting similar chromatographic profiles and retention times of the peak apex are grouped together as belonging to the same component (Figure 2). Masses with different peak shapes are grouped together as belonging to the same component (Figure 2). Masses with different peak shapes

propagated to all the laboratories with the confidence that the retention times will be the same on all the systems. This also makes it easy to review and, if necessary, re-process data to verify findings.

Figure 1 illustrates the reproducibility of retention times with and without implementing RTL. The teal line shows the daily retention time noted for cocaine over a period of a month on three instruments using RTL. The dotted blue lines separate the data from one instrument to the next. The blue line shows the daily retention time noted for cocaine over a similar time period without the use of RTL. Note the changes in retention from instrument to instrument presented by the blue line. The teal line shows a constant retention time over the same period between instrumentation. Testing was performed on other compounds to demonstrate the same level of repeatability.

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or retention times are not included in the deconvolved spectrum. This automatically removes background column bleed, and can help separate and distinguish two very closely or coeluting compounds and assign the associated masses for each of the components. In addition, the ability to include retention time in the match factor allows the software to distinguish between analytes that have similar mass spectra, but different retention times (Figure 3). The images in Figure 3 show the differences in retention time of these three isomers. The use of DRS provides a more objective approach to identification, minimizing subjectivity on behalf of the bench chemist. It also increases efficiency as deconvolution performs many of the processes typically manually executed by the forensic chemists, such as spectral subtraction.

In addition to the increase in efficiency and objectivity, benefits for the laboratory network include the ability to share mass spectral databases where the retention times are essentially the same at each of the remote sites and on each individual GC–MS system. This shared database alleviates the need to acquire, run, and measure retention times of suspected controlled substances to determine the specific isomeric form. This, in turn, provides better turnaround time in reporting of results and saves individual laboratories the cost of obtaining standard reference material. As such, master databases can be built and managed at the corporate home laboratory and periodically updated and distributed to remote sites.

An example of how RTL assisted in distinguishing para-methylacetylfentanyl and ortho-methylacetylfentanyl is described. A sample was submitted for identification and was tentatively identified as methylacetylfentanyl based on a mass spectral database search. The only certified reference material available at the time of purchase was para-methylacetylfentanyl. Upon analysis, the unknown sample was found to have a retention time of 7.833 min. The retention time of the acquired para-methylacetylfentanyl standard eluted at 7.922 min, a 0.089-min difference. While a difference of 0.089 min may not seem like a large shift, as the method is retention time locked, the change in retention time indicated a misidentification. When looking at para-methylacetylfentanyl, there is the ability for the compound to have positional isomeric species in the form of ortho- and meta-methylacetylfentanyl. A standard of ortho-methylacetylfentanyl was later purchased and analyzed with the RTL locked method. The new standard eluted at exactly 7.833 min, the identical retention time of the unknown (Figure 4). Figure 4 is an overlay of the case sample, ortho-methylacetylfentanyl, and para-methylacetylfentanyl. RTL was crucial in making this identification as the mass spectral data of these para- and ortho-isomers are identical, supporting the need for chromatography to aid in these identifications.

**Conclusion**

RTL and DRS coupled with AMDIS have been demonstrated to be valuable tools for analyzing complex seized drug materials. RTL ensures precise retention times over time and across multiple instruments. This improves the match factor, especially for chromatographically separated isomers, when combined with DRS, as shown for methylethcathinone. The combination of RTL and DRS also ensures accurate identification of unknown samples, as seen with the fentanyl derivates. Due to the ever-increasing number of compounds found in seized drugs, this approach provides a more complete analysis for identification and detection.

For Forensic use

**References**


**Francis Diamond** is a senior staff scientist for the Center for Forensic Science Research & Education (CFSRE) in Pennsylvania, USA. Previously, Diamond was Technical Director in the Criminalistics chemistry division of NMS Labs, Pennsylvania, USA, working for NMS Labs for 43 years. His areas of expertise are in the techniques of separation and detection of drugs/controlled substances.

**Abbey Fausett** is an applications chemist for the Gas Phase Separations Division within Agilent Technologies. In her 10 years with the company, she has contributed to the company by taking roles in service, support, and marketing.

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Controlling Retention Time Drift in Industrial Chromatography

Brian Rohrback, Infometrix Inc., Bothell, Washington, USA

In this instalment of The LCGC Blog, Brian Rohrback illuminates the industrial side of the chromatography application space.

Everyone who is reading this column is interested in chromatography, and I want to shine a light on the industrial side of the application space.

For argument sake, I will outline two 80/20 observations that apply to chromatography:

- 80% of published applications are for research and development (R&D) studies, and 20% are for routine applications;
- 80% of all chromatographs sold are for routine work, and 20% go to the R&D labs.

That is, of course, not surprising, but it means that we, as chromatographers, often ignore issues that are critical in routine, industrial settings that should see more of our light. So, think about it: Most chromatographs are tasked on day one with a specific analysis and, 20 years later, their final analysis will be the same as the first.

Deploying a chromatograph in a quality control (QC) or process setting is critical to many complex streams in that it provides detailed information on the composition of a huge array of mixtures and provides the means of integrating compositional control strategies in the plant. Day-to-day and instrument-to-instrument variability will frustrate data analysis. Computer-enabled
technology can be applied to mitigate the impact of ageing instruments and instrument differences, which, in turn, lead to a more robust, lower-maintenance world for industrial chromatographs. The key issue is to control retention time drift. Because peak retention on the column has some variability, this shifting increases the likelihood of misidentifying a peak and, ultimately, risks misreporting a component’s concentration. Chromatographic retention time drift from run to run is always present, but usually is not a significant source of variability. Over time, however, any retention drift amplifies the risk of an error in peak identification, which can lead, in turn, to mistakes in peak tables or in evaluating the overall distribution. Software control of retention time drift is a generic solution to this problem and addressing retention time variation simplifies ownership of a chromatograph by making the data consistent from one month to the next, even one instrument to the next. So, let’s look at software’s role in automating the handling of peak retention times as drawn from the world of chemometrics.

The literature is filled with references, but I restrict my list to two oldies, one drawn from over 50 years ago, and one from more than 20 years ago. In 1965, Kováts described how you can use peaks that are easily identified as markers to calculate relative retention times of neighbouring peaks (1). In the late 1990s, groups worked to address the problem of retention time variability by borrowing a multivariate technology that had been applied to voice recognition; one example is Correlation Optimized Warping (COW) described by Nielsen et al. (2). Software, both commercial and freeware, has been available for these approaches since the early 2000s. My team has employed both techniques, separately and in combination, for two decades or so, and has used alignment technology to manage a variety of routine and complex applications, including clinical analysis for the Centers for Disease Control (CDC), systems for economic fraud investigations for the U.S. Food and Drug Administration (FDA), and process hydrocarbon evaluations in refineries and chemical plants.

Figure 1 shows an overlay of the same sample run many times in a three-year period. Because the retention times of peaks move, the traditional way of handling this situation is to rerun the standard and accept new time positions for the peaks. This approach gives a very short-term context and, by forcing frequent recalibrations, lowers the number of samples that can be processed. Hands-on recalibration makes the system more unreliable online, where it is harder to attend to these changes. So, we often relegate this type of analysis to a supervised laboratory rather than placing it in a less-supervised position near the sampling point, where the feedback would be timely. But what if we could rely on retention times not changing? This is an example of where chemometric alignment technology should be used. For software alignment to work, one chromatogram must be previously chosen to be the alignment standard; the algorithm then adjusts the peak positions in all the other traces to match that standard as closely as possible.
Results of this process are seen in Figure 2. After alignment (here using COW) and comparing to Figure 1, we can easily see that consistent peak retention time is achieved.

COW-based alignment does not require the alignment standard to be run close to the analysis date, nor does it even need to be run on the same chromatograph. In practice, alignment keeps the peaks in place for a significantly longer time and reduces the work necessary to keep the system calibrated.

The idea is very powerful—that a small amount of software can be added to a chromatographic data system and have that addition open a true plug-and-play capability for the instrument and application. Ostensibly, a company could keep a cold spare chromatograph in inventory. When a process or laboratory unit goes down for any reason, the storage unit would be placed online and have its data be completely comparable to all previous runs on its very first injection, possibly without ever performing a traditional calibration. Alignment can also apply this procedure to the historical chromatographic data assembly, resulting in a consistent database ready to be mined.

Of course, this conclusion holds for situations where the column and the method conditions are similar. But if this is the case, the ability to perform more complex chromatographic evaluations in a fully automated manner is achieved.

References

This blog is a collaboration between LCGC and the American Chemical Society Analytical Division Subdivision on chromatography and separations chemistry.

Brian Rohrback is the President and CEO of Infometrix, the dominant independent supplier of chemometrics technology to analytical instrument companies, process analyzer suppliers, and their customers. In industry, he has held positions as a research scientist managing the chromatography group, as an exploration geologist, and as an exploration manager for Europe, Africa, and the Middle East. He has served on the board of directors of several instrument companies and consults for pharmaceutical companies, oil companies, and service organizations. Rohrback holds a B.S. in chemistry (Harvey Mudd College, California, USA), a Ph.D. in analytical chemistry/geochemistry (UCLA), and an MBA (University of Washington, USA). His publications cover topics in petroleum exploration, chemical plant optimization, clinical and pharmaceutical diagnostics, informatics, pattern recognition, and multivariate analysis.

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What is the FASTest way of getting people together and discussing science in these COVID times? Supported by a scientific committee formed by the network of tenure-track assistant/associate professors in the broad field of analytical chemistry in The Netherlands, the Forum for Analytical Science and Technology (FAST) is an important annual gathering for the exchange of the latest achievements in analytical science and technology. This article reviews previous FAST forums, and looks ahead to what’s on offer from FAST’s series of virtual e-lectures and discussions in 2021.

The Dutch COAST initiative has organized very successful meetings focusing on analytical chemistry since 2017. During “normal times” these meetings, branded under the name Forum for Analytical Science and Technology (FAST), have always been two-day, in-person gatherings. FAST creates a unique environment where academics, industrial representatives, and students meet and interact to discuss their scientific breakthroughs.

Not surprisingly, as a result of the COVID-19 pandemic, it has been impossible to organize live FAST meetings in the past year. To compensate—at least in part—for this hiatus, COAST started a series of FAST electronic science talks (FASTest), largely mimicking the intended programme of the live FAST meetings. Rather uniquely, the organization of FAST and FASTest has from the start been supported by a scientific committee formed by the network of young tenure-track assistant/associate professors in the field of analytical chemistry in The Netherlands.

The first FASTest lecture was held on Thursday 10 September 2020. During that inaugural lecture, Professor Theodore Alexandrov (European Molecular Biology Laboratory [EMBL], Germany) presented his work on spatial metabolomics. Subsequent meetings were held every two to three weeks, often centred around an overarching theme. In the 12 FASTest meetings held to date, presentations of PhD candidates, academic staff members,
and scientists from industry—both from the Netherlands and abroad—were featured, and sparked lively discussions.

One of the most recent FASTest meeting in this series was held on Wednesday 3 March 2021. The focus of the meeting was protein characterization. The opening lecture was provided by Iro Ventouri, a PhD candidate at the University of Amsterdam, The Netherlands. She showcased her work related to probing protein stability and association equilibria using asymmetrical flow field-flow fractionation (AF4). In her work she focused on an important industrial enzyme, β-D-galactosidase. The active form of this protein is a noncovalent tetramer. Association equilibria were studied in various carrier solutions, proving that the analytical conditions significantly impact the obtained result. However, when the right conditions are selected, she confirmed that AF4 preserves the labile dynamic equilibria and allows in-depth characterization of these processes.

The next speaker was Arif Arrahman, a PhD candidate at the Vrije Universiteit Amsterdam, The Netherlands. His work focuses on the characterization and activity profiling of snake venom components. He makes use of reversed-phase liquid chromatography (LC) to separate proteins and peptides in snake venom. These are subsequently collected onto 96-well plates with the aid of high-resolution fractionation. He showed that they can evaluate the activity of individual toxins in relevant bioassays. Moreover, by combining this information with proteomics data from the same fractions as well as acute and non-lethal toxicity data from behavioural zebrafish assays, they developed a truly integrated approach to evaluate snake venom function and complexity.

The session was closed by Ljiljana Paša-Tolić. She gave an overview of the efforts towards proteomics at single cell resolution within the Pacific Northwest National Laboratory (PNNL), Washington, USA. These single cell measurements are needed to decipher mechanisms that give rise to phenotypic diversity and bridge the critical gap in linking molecular-scale information to systems-level understanding of biology. Limited size and volume of single cells, together with the low copy numbers of certain proteins, make their characterization difficult. She showed the recent developments of her group towards near single cell nanodroplet sample (nanoPOTS)-based proteomics to map proteins and proteoforms comprehensively and sensitively to specific cells and within tissues. She also discussed strategies to broader disseminate near single cell proteomic technologies for assessment of molecular basis of cell-to-cell heterogeneity and spatially resolved measurements with enhanced sensitivity, throughput, and ease of operation. Overall, a great and inspiring talk to close off this FASTest meeting.

So, what is the FASTest way of getting people together to discuss science in COVID times? Make a programme that speaks to the broader community, have enthusiastic (young) speakers eager to share the latest developments, and make ample space for discussions. And if you—on top of that—make the younger generation responsible for the organization and the questions, these meetings are a guaranteed success. Do you want to join our next FASTest meeting? It is held on 19 May at 16:00 CET with "Food Analysis" as the topic. More information about the programme can be found on https://ti-coast.yvent.nu/en/. Hope to see you there!

For more information and registration, visit the COAST events website at https://ti-coast.yvent.nu/en/ E-mail: isabelle.hulshof@ti-coast.com
The 14th Congress of the French-speaking Association for Separative Sciences (SEP21) will take place at the Porte de Versailles in Paris, France, from 5–7 October 2021. This preview explores what visitors can look forward to from the conference.

The objective of SEP21 is to bring together major players in the field of separation sciences from industry, academia, and private laboratories. This conference will offer an opportunity to discuss recent trends, to discover new areas of research from both a theoretical and application point of view, and to exchange experiences. The scientific programme will include plenary lectures by internationally renowned scientists, oral presentations, and poster sessions. The opening session will include a presentation by Jean-François Focant (Liège University, Belgium) and the closing session will feature Caroline West (Orléans University, France).

SEP21 will cover many aspects: new technologies, instrumentation, data processing (chemometrics and quality-by-design [QbD]), analytical development, all modes of chromatography and electrophoretic separation techniques, in addition to detection methods such as mass spectrometry and sample preparation across all applications of analytical sciences, including pharmaceuticals and cosmetics. Ten awards will be given for the best oral and poster presentations for young researchers, and three lunch-time seminars will be presented by exhibitors. You will be able to follow these presentations (approximately 30 min) while enjoying your lunch, and boxed lunches will be provided to all participants. A roundtable discussion between editors and authors will be held with the editors of publications including Chromatographia, Journal of Separation Science, and Journal of Chromatography B, and articles linked to the symposium presentations may be submitted for publication in the Journal Analytical and Bioanalytical Chemistry.

Besides this attractive scientific programme, the committee has organized a special French dinner to delight your
taste buds, and which will also present an opportunity to exchange ideas and build new relationships.

Before the congress, four short e-learning courses will be given on Friday 1 October (morning and afternoon) on the following topics:
• From Acquisition to Data: Analytical and Methodological Aspects in Metabolomics (Part 1), by Julien Boccard (Centre Médical Universitaire, Geneva, Switzerland) and Serge Rudaz (University of Geneva, Geneva, Switzerland).
• From Data to Biological Information: Statistical and Bioinformatics Aspects in Metabolomics (Part 2), by Julien Boccard and Serge Rudaz.
• Hyphenation of Separative Methods to Mass Spectrometry for the Analysis of Therapeutic Proteins: Technical Possibilities, Applications, and Perspectives, by Arnaud Delobel (Quality Assistance, Donstiens, Belgium), Davy Guillarme (University of Geneva, Geneva, Switzerland), and Yannis François (University of Strasbourg, France).
• From Development to Measurement: Uncertainty, Methodology, and Tools for the Industry/Analyst, by Jean-Marc Roussel (Consultant in analytical chemistry and validation, Mâcon, France) and Dominique Sotty (Servier, Orléans, France).

Please note, this will be a French-speaking event and the programme will be presented as such. With such a diverse and high-quality programme, we are confident there will be something for everyone to enjoy and we very much look forward to welcoming you in Paris for SEP21!

For more information and registration, visit the SEP21 webpage at www.sep2021.fr
E-mail: info@sep2021.fr
HTC-17 Preview

Deirdre Cabooter, University of Leuven. Leuven, Belgium

The 17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-17) will be held in person from 26 to 28 January 2022, at Conference Center Het Pand in Ghent, Belgium. HTC-17 will be organized under the auspices of the Royal Flemish Chemical Society (KVCV) and the Separation Science Group of the Royal Society of Chemistry (SSG RSC).

The HTC conference has been the premier platform for state-of-the-art developments in separation technologies and hyphenated techniques for more than 30 years. The conference typically encompasses three parallel sessions consisting of plenary lectures, keynote lectures, tutorials, oral, and poster-flash presentations. One of the parallel sessions will be dedicated to young emerging scientists (YES!). The symposium will also host an attractive technical exhibition where vendors will present their newest instruments and developments, topped with technical seminars.

We will cover fundamental and practical aspects of liquid-phase (LC) and gas chromatography (GC), including multidimensional LC, multidimensional GC, supercritical fluid chromatography (SFC), LC–mass spectrometry (MS), and GC–MS. The programme will include topics such as three-dimensional separations, automated sample preparation, online analyzers and sensors, ion-mobility and native mass spectrometry, emerging detectors and separation modes, method development and artificial intelligence, column technology and stationary-phase developments, system design and optimization, miniaturization and chip technology, and data mining and curation. We will also address bottlenecks and describe trends and new technologies for a wide range of applications, including (bio-)pharmaceuticals, macromolecules, medical diagnostics and clinical applications, forensic analysis and doping control, food analysis and safety, environmental analysis, energy, green approaches, high-throughput analysis, -omics (lipidomics, metabolomics, proteomics), and biomarker discovery, as
well as modern industrial applications and natural products.

As always, we will ensure abundant networking opportunities during the conference, with an informal beer tasting event organized during a nocturnal poster event, a conference dinner in the beautiful PoortAckere monastery, and lots of dissemination activities.

A number of awards will be presented at HTC-17. The HTC/LCGC Innovation Award, sponsored by LCGC Europe, will be offered to a researcher with less than 15 years’ experience after obtaining his or her PhD for “a pioneering contribution to the field of separation sciences by introducing new methodologies, new instrumentation, or new techniques in the field, with a strong focus on applicability”. The most innovative oral contribution presented during the conference will receive the HTC-Award. The most innovative poster contributions will receive the HTC-Poster Award. We will also organize a YouTube contest for young scientists that will be sponsored by Elsevier’s Journal of Chromatography A and Journal of Chromatography Open, a new open access journal.

Participants are invited to register and submit abstracts for review using the abstract submission menu available on the website: www.htc-17.com. The deadline for oral abstract submission is 15 September 2021. The poster abstract submission deadline is 15 October 2021.

After a challenging time wherein meeting and networking has been severely restricted, we very much look forward to seeing you in Ghent!

For more information and registration, visit the HTC-17 webpage at: https://htc-17.com/
E-mail: htc17@kuleuven.be
### Training Courses

**GC**
- **GC Introduction**
  - **Website:** [www.chromacademy.com/](http://www.chromacademy.com/)channels/gc-training-courses/principles/gc-introduction

**GC Troubleshooter**
- **Website:** [www.chromacademy.com/](http://www.chromacademy.com/)channels/gc-training-courses/troubleshooting/gc-troubleshooter

**GC Fundamentals**
- **Website:** [www.crawfordscientific.com/training-consultancy/gc-training/gc-fundamentals](http://www.crawfordscientific.com/training-consultancy/gc-training/gc-fundamentals)

**Complete GC & GC–MS**
- **From 21 May 2021**
  - The Open University, Milton Keynes, UK, or online
  - **Website:** [www.anthias.co.uk/content/2-day-gc-gc-ms-clinic-1](http://www.anthias.co.uk/content/2-day-gc-gc-ms-clinic-1)

**The GC & GC–MS Clinic**
- **9–10 June 2021**
  - The Open University, Milton Keynes, UK, or online
  - **Website:** [www.anthias.co.uk/content/5-day-complete-gc-gc-ms](http://www.anthias.co.uk/content/5-day-complete-gc-gc-ms)

**HPLC/LC–MS**
- **Absolute Basics of HPLC and LC–MS**
  - **25 May 2021**
  - The Open University, Milton Keynes, UK, or online
  - **Website:** [https://www.anthias.co.uk/training-courses/AB-LC](https://www.anthias.co.uk/training-courses/AB-LC)

**HPLC Fundamentals**
- **Onsite training**
  - **Website:** [www.crawfordscientific.com/training-consultancy/hplc-training/hplc-fundamentals](http://www.crawfordscientific.com/training-consultancy/hplc-training/hplc-fundamentals)

**HPLC Troubleshooter**
- **Website:** [www.chromacademy.com/channels/hplc-training-courses/troubleshooting/hplc-troubleshooter](http://www.chromacademy.com/channels/hplc-training-courses/troubleshooting/hplc-troubleshooter)

**Fundamental LC–MS**
- **Website:** [www.chromacademy.com/channels/lc-ms/principles/fundamentals-of-lc-ms-video-training-course](http://www.chromacademy.com/channels/lc-ms/principles/fundamentals-of-lc-ms-video-training-course)

**LC–MS Introduction**
- **Onsite training**
  - **Website:** [www.chromacademy.com/channels/lc-ms/principles/lc-ms-introduction](http://www.chromacademy.com/channels/lc-ms/principles/lc-ms-introduction)

**SAMPLE PREPARATION**
- **Fundamentals of Solid-Phase Extraction (SPE) Mechanisms**
  - **Online training**

**MISCELLANEOUS**
- **Coping With COVID-19: Remaining Productive and Safe in the Analytical Laboratory**
  - **Online webcast from CHROMacademy**

Please send your event and training course information to Kate Jones
KJones@mjhlifesciences.com

**Introduction to Infrared (IR) Spectroscopy**
- **Online webcast from CHROMacademy**
  - **Website:** [www.chromacademy.com/channels/infrared/principles/introduction-to-infrared-spectroscopy](http://www.chromacademy.com/channels/infrared/principles/introduction-to-infrared-spectroscopy)

**Applied Size-Exclusion Chromatography (SEC)**
- **8 June 2021**
  - The Open University, Milton Keynes, UK, or online
  - **Website:** [www.anthias.co.uk/training-courses/Applied-Size-Exclusion-Chromatography](http://www.anthias.co.uk/training-courses/Applied-Size-Exclusion-Chromatography)

**Absolute Basics of Chemometrics**
- **28–29 June 2021**
  - The Open University, Milton Keynes, UK, or online
  - **Website:** [www.anthias.co.uk/training-courses/basics-chemometrics](http://www.anthias.co.uk/training-courses/basics-chemometrics)
# Event News

## 29 June–2 July 2021

**26th International Symposium on Separation Sciences (ISSS 2020) and the 25th International Symposium for High-Performance Thin-Layer Chromatography (HPTLC 2020)**

Grand Hotel Union, Ljubljana, Slovenia  
**E-mail:** info@issss2020.si and info@hptlc2020.si  
**Website:** https://issss2020.si/ and https://hptlc2020.si/

## 5–7 October 2021

**Forum Labo**  
Paris Expo Porte de Versailles, Paris, France  
**E-mail:** infos@forumlabo.com  
**Website:** https://www.forumlabo.com/paris/en-gb.html

## 2–5 November 2021

**The 10th International Symposium on Recent Advances in Food Analysis (RAFA 2021)**  
Clarion Congress Hotel, Prague, Czech Republic  
**E-mail:** RAFA2021@vscht.cz  
**Website:** http://www.rafa2021.eu

## 26–28 January 2022

**The 17th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-17)**  
Het Pand Conference Center, Ghent, Belgium  
**E-mail:** htc17@kuleuven.be  
**Website:** https://htc-17.com/

## 11–14 October 2022

**The 18th International Symposium on Preparative and Industrial Chromatography and Allied Techniques (SPICA 2022)**  
Lisbon, Portugal  
**E-mail:** secretariat@LDOrganisation.lu  
**Website:** https://www.spica2022.org/
## Contact Information

**Europe**

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