Evaluation of Enzyme-Linked ImmunoSorbent Assay and Liquid Chromatography-Tandem Mass Spectrometry as Screening and Confirmation Methods for the Detection of Synthetic Cannabinoids

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Abstract

Synthetic cannabinoids (SCs) have started to become a worldwide drug epidemic since their adaptation to the mainstream drug market in 2008. Numerous detection techniques were employed after the initial realisation that it was a chemical compound, not the innocuous plant matter which provided the psychoactive properties for products such as Spice or ‘K2’. The results indicated a wide range of synthesised homologues designed around the stimulation of the cannabinoid receptors. However, these results were only able to be achieved at the time through the use of instruments such as liquid-chromatography-tandem mass spectrometry (LC-MS/MS) which were not available to all facilities. A cheaper and simple system which still maintained accurate and precise quantitative results was needed to prevent this growing problem.

The initial stage of the research project involved the evaluation of two enzyme-linked immunosorbent assay (ELISA) kits; the SC 3474 kit from Randox Toxicology and the K2 direct ELISA kit by Immunalysis. The chosen SC standards included 18 parent drug and metabolite homologues with some presenting constitutional isomerism. Analysis established the creation of a percent cross-reactivity (%CR) profile, using the acquired standards compared to each ELISA's antibody. For Randox Toxicology, 10 of the 18 SC standards returned %CR scores greater than 40%. However, only five standards returned %CR greater than 40% using the Immunalysis kit, with six returning no value at all.

The following stage implemented a comparative analysis using LC-MS/MS. The LC-MS/MS method included the development of optimised multiple reaction monitoring (MRM) conditions specific for each SC analogue. Subsequent LC conditioning incorporated the use of a gradient elution program for separation of problematic analytes whilst providing a rapid elution for high throughput and resolution. The experimental results using the LC-MS/MS method demonstrated the identification and quantification of all 18 SC analogues. The method was validated with linearity, inter-assay accuracy, precision specificity, and carryover effect, all presenting results within acceptable ranges.

Furthermore, the robustness of both detection techniques was evaluated with the addition of two common adulterants; oleamide and α-tocopherol. The impact on the ELISA was determined both without and with the presence of SCs, measuring a change in each analytes %CR score. Despite the impact of the adulterants on the ELISAs not
being able to be quantified, a definite alteration was witnessed. The specificity of the LC-MS/MS method was evaluated by a change in the accuracy of the SC analyte, and whether it remains within tolerable bias. With the applied method, it was concluded that all 18 SC standards remained within this tolerable range.

The dual assessment of these two techniques allowed for the determination of an ELISA’s ability to be used as a replacement of, or in compliment with, a valid LC-MS/MS method. However, due to the presumptive nature of ELISA analysis, it was concluded that this analysis is acceptable only as a screening method, in conjunction with a confirmatory test such as LC-MS/MS when required.
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### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
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<tbody>
<tr>
<td>%CR</td>
<td>Percent cross-reactivity</td>
</tr>
<tr>
<td>%CV</td>
<td>Percent coefficient of variance</td>
</tr>
<tr>
<td>#HI</td>
<td>#-hydroxyindole metabolite</td>
</tr>
<tr>
<td>#HP</td>
<td>N-(#-hydroxypentyl) metabolite</td>
</tr>
<tr>
<td>AAI</td>
<td>Aminoalkylindole</td>
</tr>
<tr>
<td>ADR</td>
<td>Adverse drug reaction</td>
</tr>
<tr>
<td>AM-</td>
<td>Alexandros Makriyannis</td>
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<tr>
<td>CB₁</td>
<td>Cannabinoid receptor type 1</td>
</tr>
<tr>
<td>CB₂</td>
<td>Cannabinoid receptor type 2</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSA</td>
<td>Controlled Substances Act</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>HHC</td>
<td>(-)-9-nor-9b-hydroxyhexahydrocannabinol</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>JWH-</td>
<td>John W. Huffman</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LLE</td>
<td>Liquid-liquid extraction</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
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<tr>
<td>-MS/MS</td>
<td>-Tandem mass spectrometry</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>O</td>
<td>Oleamide (with L/M/H, denoting low, medium or high)</td>
</tr>
<tr>
<td>PA</td>
<td>N-pentanoic acid metabolite</td>
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<tr>
<td>SAR</td>
<td>Structure activity relationships</td>
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<tr>
<td>SC</td>
<td>Synthetic Cannabinoids</td>
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<tr>
<td>SIM</td>
<td>Selective-ion monitoring</td>
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<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
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<tr>
<td>SUSMP</td>
<td>Standard for the Uniform Scheduling of Medicines and Poisons</td>
</tr>
<tr>
<td>SWGDRUG</td>
<td>Scientific Working Group for the Analysis of Seized Drugs</td>
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<tr>
<td>T</td>
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</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THC</td>
<td>Delta-9-tetrahydrocannabinol</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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