

Joana Couceiro<sup>1,2</sup>, Haider Sultan, Smilja Duranovic, Suzanne Bell<sup>3</sup> and Alexandre Quintas<sup>1,2</sup>

<sup>1</sup>Laboratório de Ciências Forenses e Psicológicas Egas Moniz, Campus Universitário - Quinta da Granja, Monte de Caparica, 2825-084 Caparica, Portugal

<sup>2</sup>Centro de Investigação Interdisciplinar Egas Moniz, Instituto Superior de Ciências da Saúde Egas Moniz, 2829-511 Monte da Caparica, Portugal

<sup>3</sup>C. Eugene Bennett Department of Chemistry, West Virginia University

## INTRODUCTION

The emergence and abuse of synthetic cannabinoids has been rising during the last years, mostly among younger populations, as an alternative to cannabis<sup>1</sup>. Given their relatively recent appearance, the pharmacological and toxicological profiles of these new psychoactive substances are not well understood. Current studies suggest that they have stronger psychoactive effects compared to natural cannabinoids and their metabolites retain affinity towards CB1 receptors in the CNS<sup>2</sup>.

In this study, the cell toxicity of JWH-018, one of the first synthetic cannabinoids appearing on the market<sup>3</sup>, and its N-(3-hydroxypentyl) metabolite was assessed using human cell lines HEK-293T and SH-SY5Y by the MTT assay. The results were compared with those obtained with the LDH assay and the Scepter 2.0 cell counter. This device is an automated handheld device that uses the Coulter principle of impedance to detect particles or cells. This implementation of the methodology enables the accurate discrimination of cell populations according to cell size and volume<sup>4</sup>.

## RESULTS

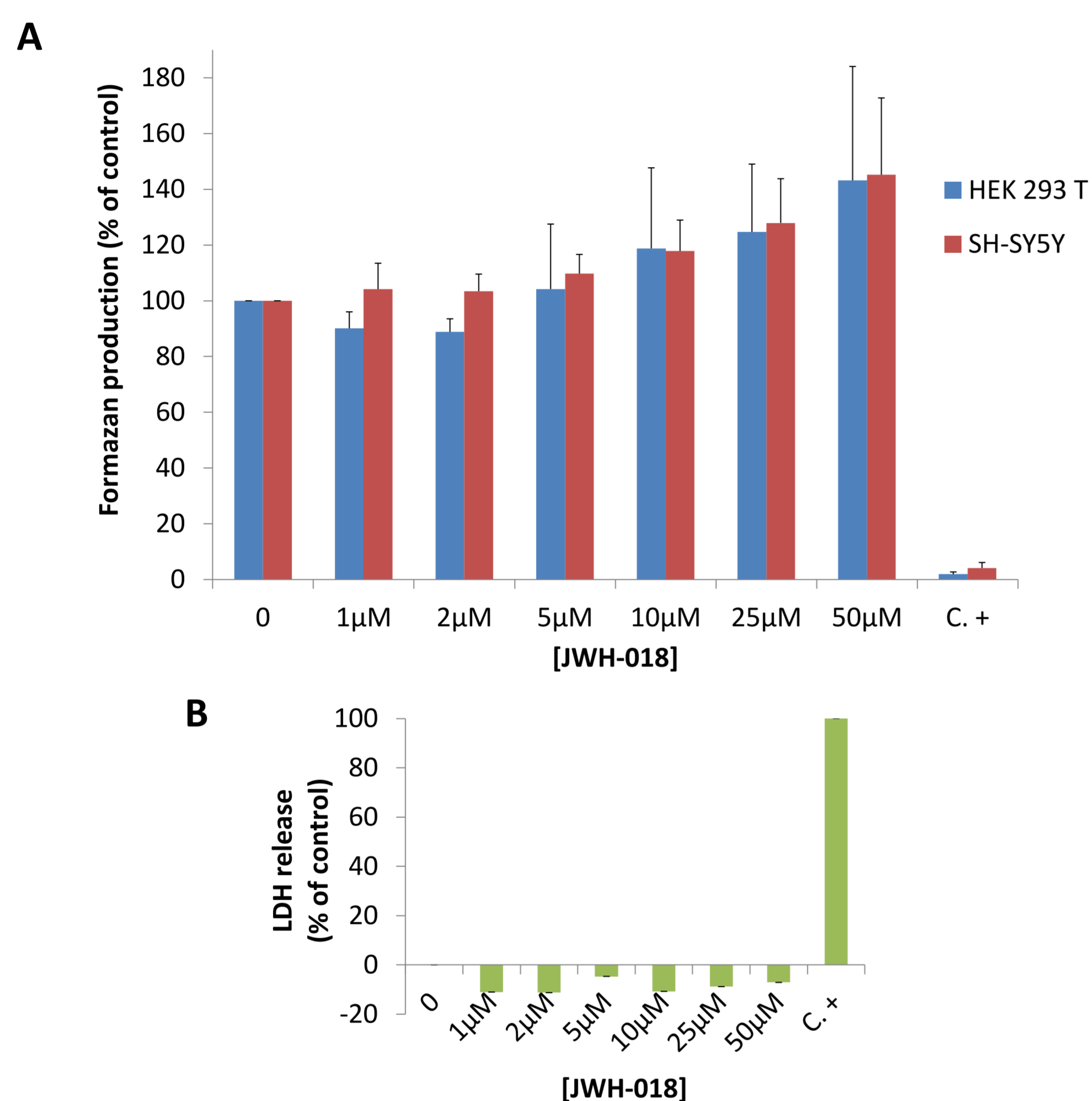


Fig. 1 - **Cytotoxic Evaluation of JWH-018 in HEK 293 T and SH-SY5Y cells.** Both sets of cells were treated with 0, 1, 2, 5, 10, 25 and 50 μM of JWH-018 for 24 h. (A) Formazan formation as determined by the MTT assay. (B) Results for the LDH assay. Data are expressed as the mean ± 1S (n = 4 or 5).

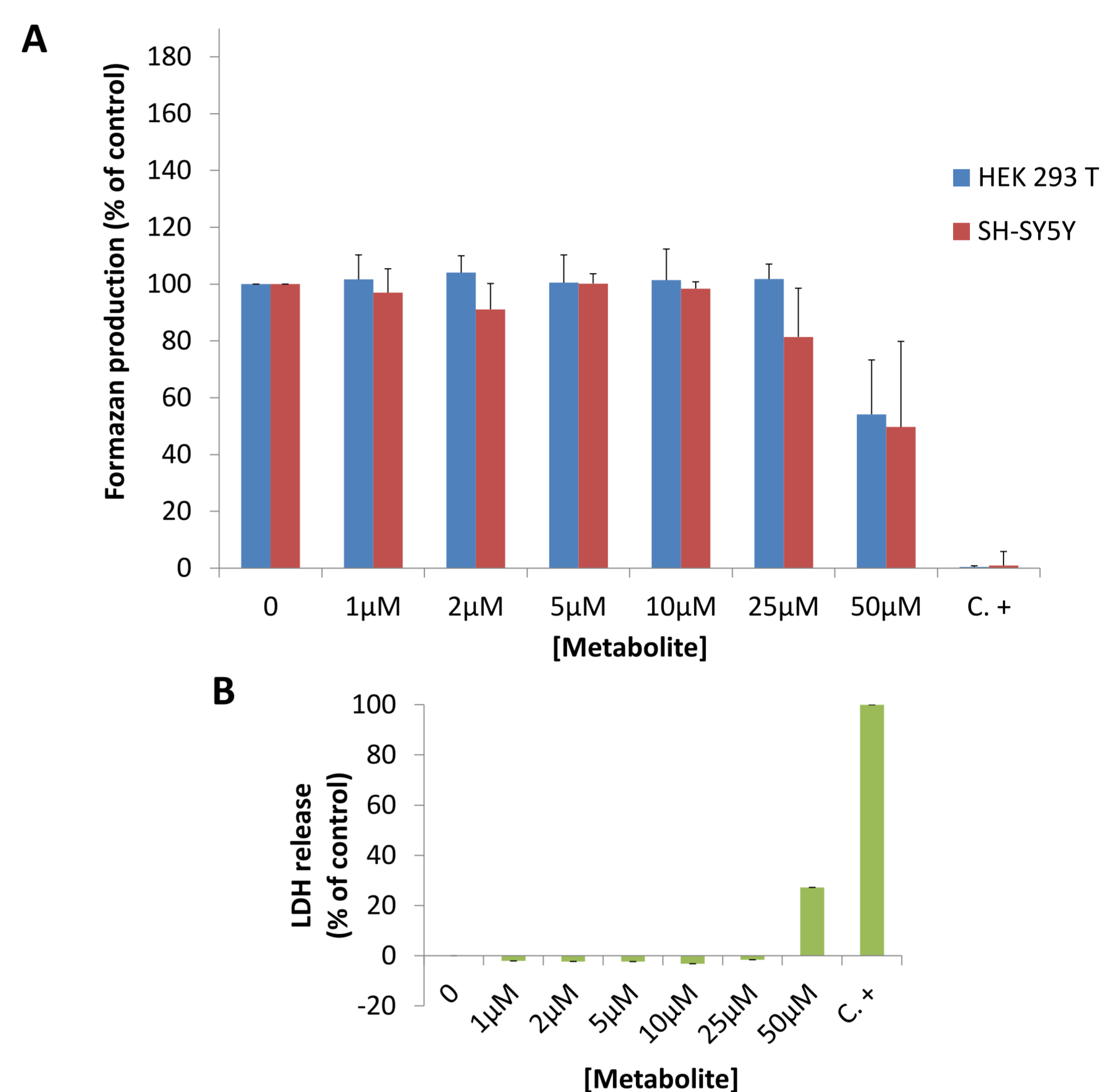


Fig. 2 - **Cytotoxic Evaluation of JWH-018 N-(3-hydroxypentyl) metabolite in HEK 293 T and SH-SY5Y cells.** Both sets of cells were treated with 0, 1, 2, 5, 10, 25 and 50 μM of JWH-018 N-(3-hydroxypentyl) metabolite for 24 h. (A) Formazan formation as determined by the MTT assay. (B) Results for the LDH assay. Data are expressed as the mean ± 1S (n = 4 or 5).

Drug exposure concentration	
0 μM	50 μM
Mean cell diameter (μm)	
(A) Cells exposed to JWH-018	
12,54 ± 0,18	12,73 ± 0,31
(B) Cells exposed to JWH-018 N-3OH-Pentyl metabolite	
12,41 ± 0,21	10,8 ± 0,2

Fig. 3 – **Mean diameter of SH-SY5Y cells exposed to (A) JWH-018 and (B) JWH-018 N-(3-hydroxypentyl) metabolite.** The SH-SY5Y cells treated with 0 and 50 μM of each drug for 24 h were analysed by the Scepter 2.0 cell counter. The diameter measurements obtained were acceptably reproducible, displaying the coefficients of variation less than 5%.

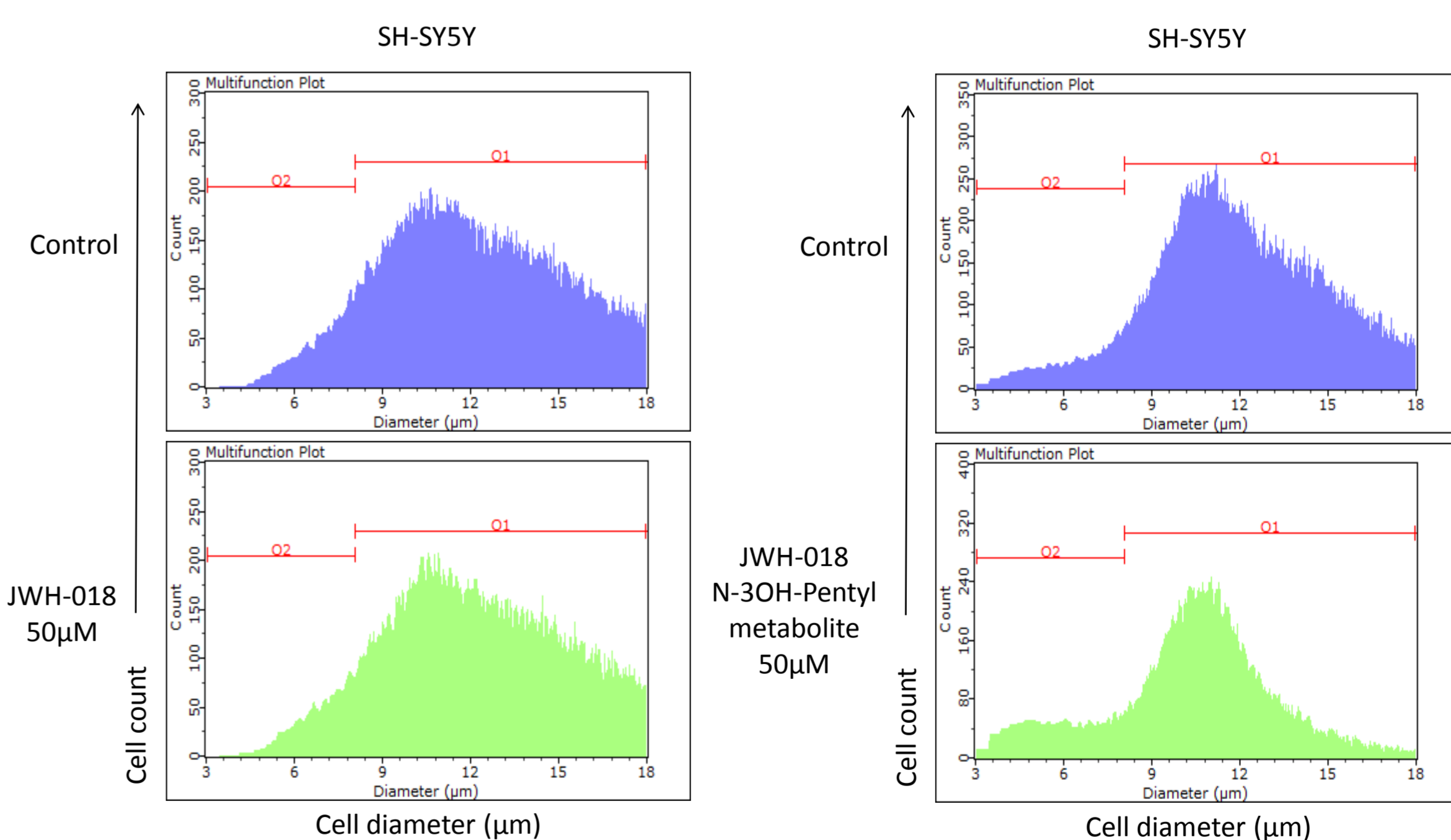


Fig. 4 – **Comparison of SH-SY5Y cell size distributions obtained with the Scepter.** Diameter distributions of SH-SY5Y cells treated with 0 and 50 μM of (A) JWH-018 and (B) JWH-018 N-(3-hydroxypentyl) metabolite for 24 h as measured by the Scepter 2.0 cell counter.

## CONCLUSIONS

The JWH-018 MTT results points to no toxicity impact of this substance on human cells until a concentration of 50 μM is reached. In fact, JWH-018 seems to increase cell growth up to concentrations of 50 μM, the maximum concentration used in our assays. These results agree generally with literature reports. On the other hand, the JWH-018 N-(3-hydroxypentyl) metabolite shows a toxicological impact on cells above 25 μM. The results of both MTT and LDH assays demonstrate the same trend. In addition, we have performed a cell volume analysis, using the Scepter 2.0 cell counter, with the aim of study JWH induced apoptosis. The preliminary results indicate that the JWH-018 metabolite induces apoptosis while the parent JWH-018 does not.

## REFERENCES

- Vandrey, R., et al. (2012) *Drug and alcohol dependence* **120**, 238–41
- Aung, M., et al. (2000) *Drug and alcohol dependence* **60**, 133–40
- European Monitoring Centre for Drugs and Drug Addiction (2009) Thematic paper — Understanding the 'Spice' Phenomenon, Luxembourg: Office for Publications of the European Communities; doi: 10.2810/27063
- Tahara, M. et al. (2013) *Biochemical and biophysical research communications* **434** 753–9