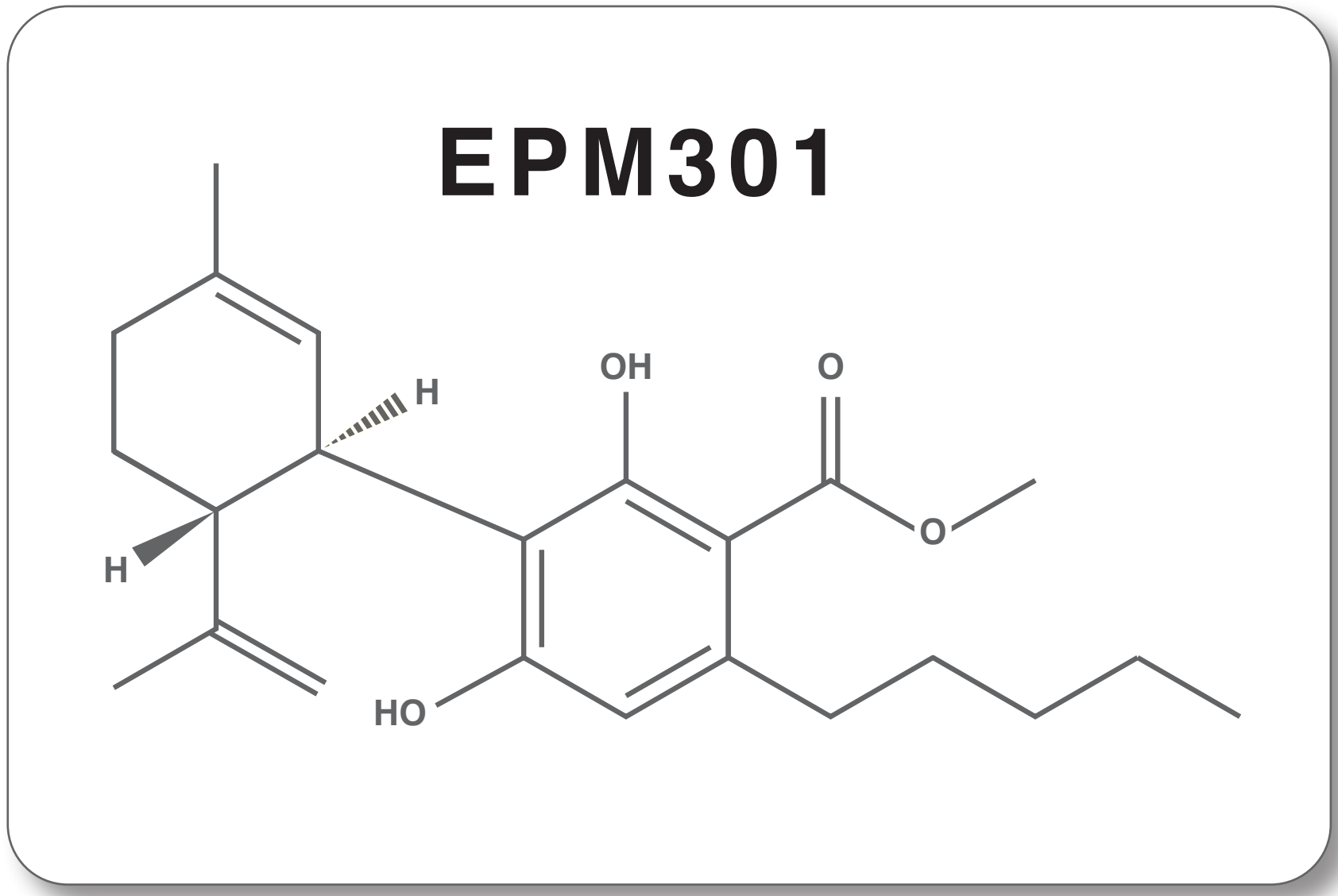


THE UNIQUE METABOLIC PROFILE OF EPM301, A NOVEL CANNABIDIOL ANALOG, IN THE TREATMENT OF PRADER-WILLI SYNDROME

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- EPM301, a novel cannabidiol (CBD) analog currently in development by EPM Therapeutics, is a promising candidate for the treatment of patients with Prader-Willi Syndrome (PWS).



- A recent study demonstrated that EPM301 (20 and 40 mg/kg/day, intraperitoneally) effectively reduced body weight and hyperphagia in high-fat diet-fed *Magel2* null mouse model of PWS.¹
- The metabolic profile of EPM301 was evaluated in both *in vitro* and *in vivo* studies to further confirm its therapeutic potential in patients with PWS.

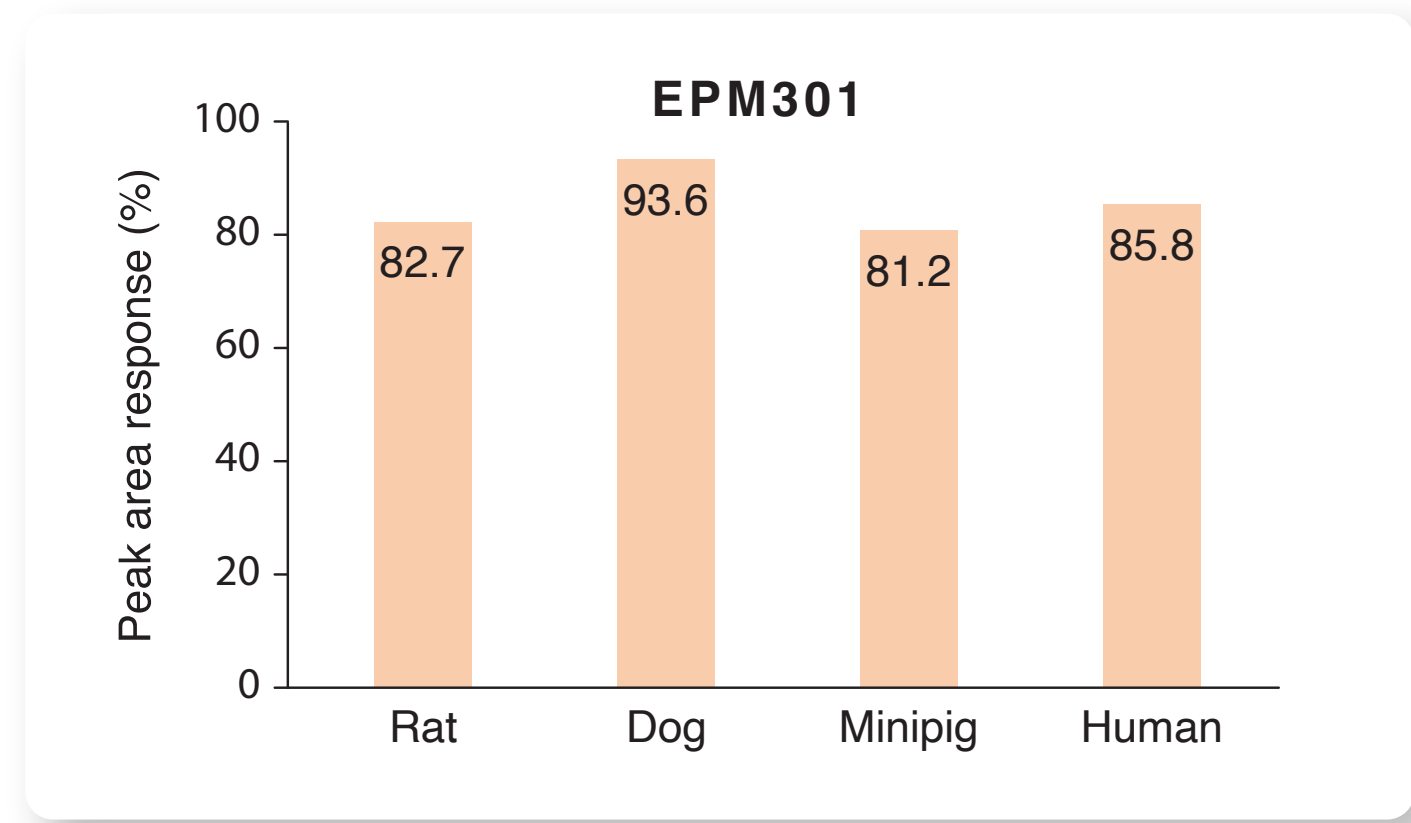
EPM301 exhibited limited metabolism *in vitro* across rat, dog, minipig, and human hepatocytes, unlike CBD²

METHODS

- An *in vitro* biotransformation study of EPM301 using rat, dog, minipig, and human hepatocytes was conducted to characterise its metabolites.
- Cryopreserved hepatocytes were incubated in a nominal concentration of 10 μ M EPM301 in suspension in Leibovitz L-15 medium for up to 4 hours.
 - To ensure suitable metabolic capacity of the hepatocytes used in the EPM301 incubations, positive control incubations with [¹⁴C]7-ethoxycoumarin were conducted.
- Samples taken at 0 and 4 hours were analysed using accurate mass LC-MS/MS to determine:
 - The relative proportions of EPM301 present at each timepoint
 - The profile of metabolites formed.

RESULTS

- Following 4 hours of EPM301 incubation, limited metabolism of EPM301 was observed in all species.
- The total peak area responses of the parent compound, EPM301, across species are shown below. The extent of metabolism was minipig > rat > human > dog.



- The most abundant metabolite, M13 (EPM301 + O), accounted for 4.0%, 0.2%, 5.0%, and 4.0% of the total peak area response in rat, dog, minipig, and human hepatocytes, respectively.
- The second most abundant metabolite, M9 (EPM301 + Glucuronide), accounted for 0.2%, 4.0%, 3.3%, and 2.6% of the total peak area response in rat, dog, minipig, and human hepatocytes, respectively.
- All human metabolites were also detected in at least one animal species.
- The *in vitro* biotransformation of EPM301 is dramatically distinct from CBD (whose major metabolites *in vitro* were 7-OH-CBD, 7-COOH-CBD, and 6-OH-CBD³).

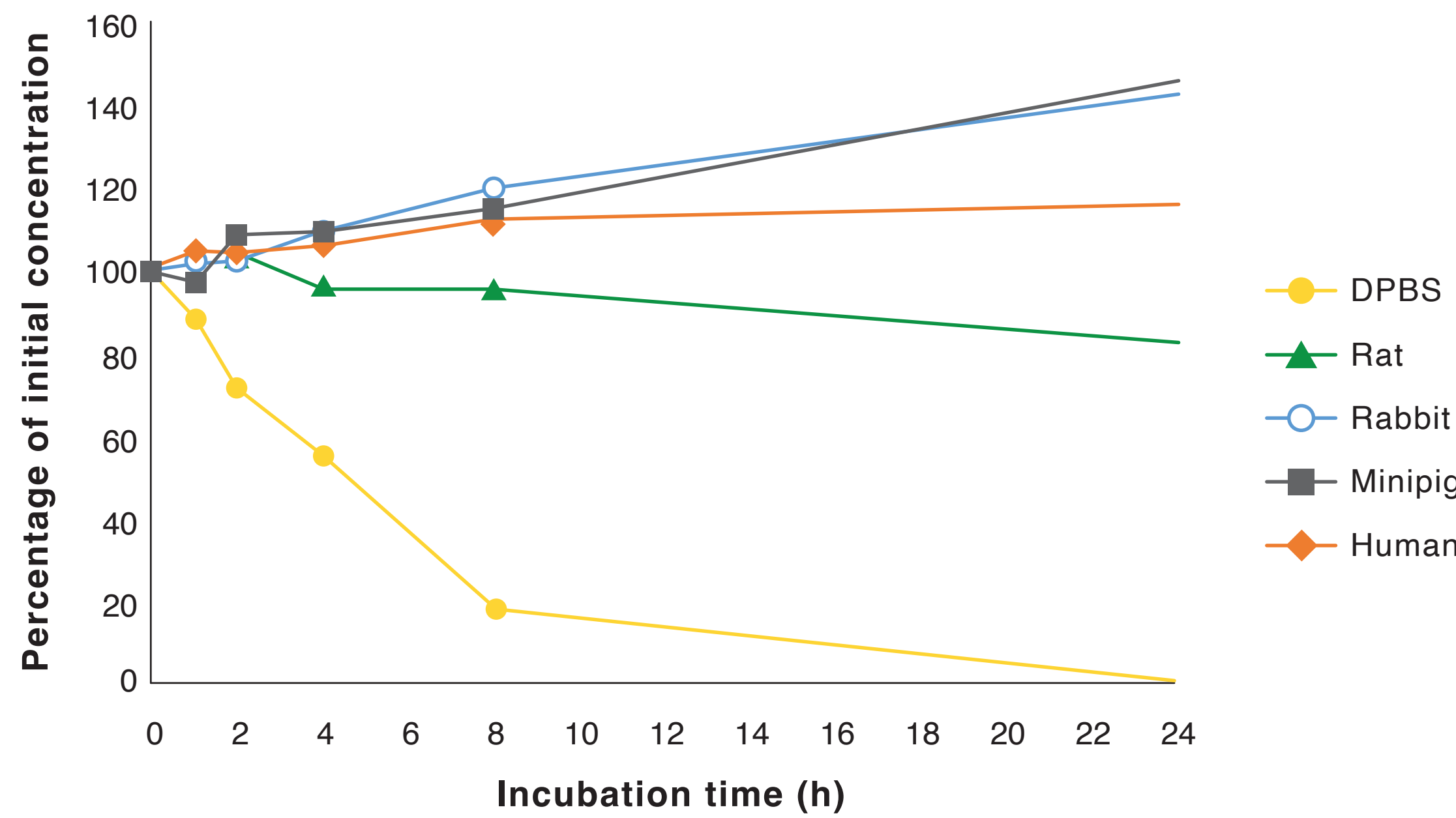
EPM301 exhibited high binding to rat, rabbit, minipig, and human plasma proteins *in vitro*²

METHODS

- An *in vitro* study using rat, rabbit, minipig, and human plasma was conducted to determine the stability of EPM301 and the extent of its *in vitro* binding to the plasma proteins.
- The stability of EPM301 (7500 ng/mL) was assessed in Dulbecco's Phosphate-Buffered Saline (DPBS) buffer, and male Han Wistar rat, female New Zealand White rabbit, male Göttingen minipig, and male human plasma.
 - Incubations were performed for 0, 1, 2, 4, 8, and 24 hours in a shaking water bath maintained at 37°C. Matrix-matched samples were then analysed using LC-MS/MS.
- Equilibrium dialysis: the duration of dialysis required to achieve equilibration of the unbound fraction was firstly determined in human plasma using the high throughput dialysis apparatus (HTD) device.
 - EPM301 at a nominal concentration of 7500 ng/mL was incubated for up to 24 hours at 37°C and 5% CO₂, and samples were dialysed for 1, 2, 4, 8, and 24 hours.
 - EPM301 concentrations were determined in the donor and acceptor compartments by LC-MS/MS in order to establish the time to equilibrium of the unbound fraction.
 - Based on the equilibration time experiment, the established time to equilibrium was 2 hours.
- Plasma protein binding: the concentration-dependent protein binding of EPM301 was determined in rat, rabbit, minipig, and human plasma samples.
 - Plasma samples were spiked with EPM301 at 7500, 10000, 15000 ng/mL, and pre-incubated at 37°C for 15 minutes.
 - Triplicate samples were then dialysed at 37°C/5% CO₂ for 2 hours (as established in the equilibration experiment).
 - The high-binding compound [14C]-warfarin (1000 ng/mL) was used as a positive control.
 - Matrix-matched plasma and buffer samples were analysed by protein precipitation, dilution of the supernatant and analysis by LC-MS/MS in MRM mode using turbo ionspray as the ionisation technique.

RESULTS

- EPM301 (7500 ng/mL) was stable in plasma from all species, following incubation for up to 24 hours at 37°C. EPM301 concentrations declined slightly in the rat plasma, accounting for 81.8% of the initial concentration after 24 hours. However, EPM301 concentrations decreased in a time-dependent manner in DPBS buffer (accounting for 0.473% of the initial concentration after 24 hours).



- Equilibration time incubations determined that a dialysis time of 2 hours was sufficient for unbound EPM301 to reach equilibrium for plasma protein binding determination, as the data at 2 hours showed good recovery values, low non-specific binding and replicate precision.
- EPM301 appeared very highly bound to male Han Wistar rat, female New Zealand White rabbit, male Göttingen minipig, and male human plasma proteins, with a mean free fraction range of 0.0102% to 0.199% across the concentration levels tested.
 - The high plasma protein binding observed suggests that the free circulating concentration of EPM301 may be limited *in vivo*.

Species	Overall mean protein bound ± SD (%)	Overall mean free fraction ± SD (%)
Rat	100 ± 0	0.0102 ± 0.0067
Rabbit	100 ± 0	0.0367 ± 0.0374
Minipig	99.8 ± 0.2	0.199 ± 0.244
Human	99.9 ± 0.2	0.120 ± 0.186

SD: standard deviation

EPM301 was the most abundant compound following oral administration in rat and minipig²

METHODS

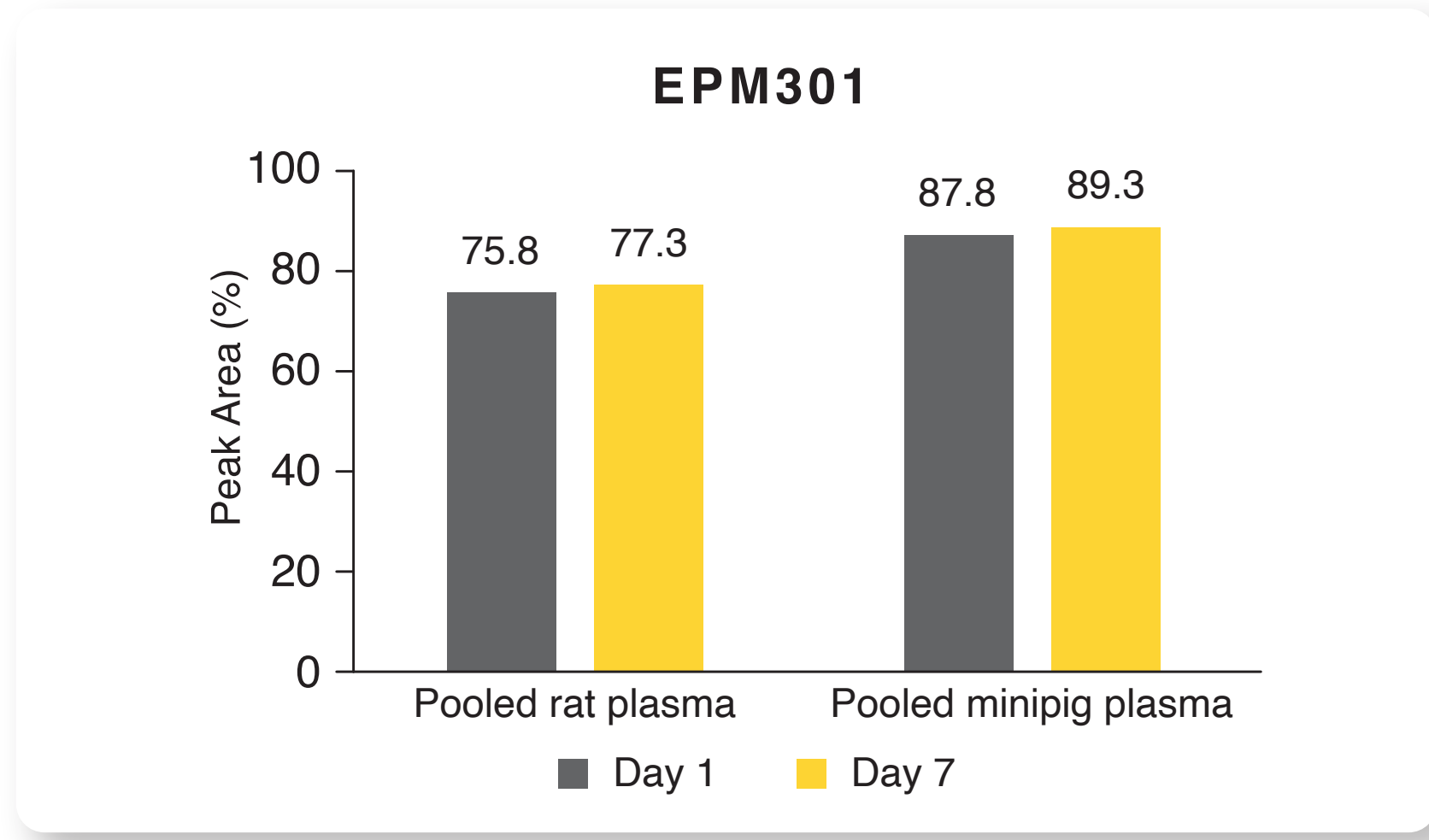
- An additional study was conducted to profile and characterise the major metabolites in the plasma samples of rat and minipig following single and repeat oral administration of EPM301 (1000 mg/kg/day).
- Selected rat and minipig plasma samples were obtained from the dose range finding studies. The selected samples were pooled across animals by species, sex and day to generate 0–24 hour area under the concentration time curve (AUC_(0-24h)) pooled plasma samples, which were then profiled using liquid chromatography-high resolution mass spectrometry (LC-HRMS).

	Fixed dose	Sex	Pooling regimes
			Day 1, AUC _(0-24h)
Rat	EPM301 1000 mg/kg/day	Male	Day 7, AUC _(0-24h)
		Female	Day 1, AUC _(0-24h)
Minipig	EPM301 1000 mg/kg/day	Male	Day 7, AUC _(0-24h)
		Female	Day 1, AUC _(0-24h)

- Day 1 pre-dose (0 hour) samples were used as controls during the LC-HRMS analysis.
- The peak area of the parent compound and potential metabolites detected in each sample were determined from the respective accurate mass extracted ion chromatogram. The peak response of each component was calculated as a percentage of the total evaluated response.

RESULTS

- The metabolite profiling data presented allow for relative comparisons only, as they were not generated using qualitative analytical methods.
- Following single and repeat oral administration of EPM301 (1000 mg/kg/day) to rats and minipigs, EPM301 and up to 18 metabolites were detected across the AUC_(0-24h) pooled plasma samples analysed.
 - The main routes of biotransformation were oxidation, demethylation, reduction, glucuronidation and sulfate conjugation.
- In both rat and minipig, EPM301, the unchanged parent compound, was the most abundant circulating component at Day 1 and Day 7. The percentage of total evaluated LC-HRMS peak area in both species are shown below.



- The most abundant metabolites in pooled rat plasma were M20, M13, and M14, and in pooled minipig plasma were M9 and M23.

	Metabolites	Total Evaluated Peak Area (%)
	M20 (di-demethylated saturated EPM301)	6.6
Pooled rat plasma	M13 (oxidated EPM301)	4.3
	M14 (demethylated EPM301)	3.4
	M9 (glucuronide conjugate of EPM301)	4.8
Pooled minipig plasma	M23 (di-oxidated and desaturated EPM301)	4.5

- All other metabolites detected across the rat and minipig AUC_(0-24h) pooled plasma samples each accounted for <2.5% of the total evaluated peak area.
- No significant accumulation was observed for most of the metabolites following repeat dosing. However, M20 in the rat and M9 in the minipig were observed at higher abundances (>2 fold) at day 7 when compared to day 1.
- Metabolite profiles were comparable across male and female for both rat and minipig, indicating no notable sex differences in the metabolites formed or their estimated abundances.

EPM301 demonstrated a distinctive and well-characterised metabolic profile across the non-clinical studies evaluated. The metabolic profile was markedly different from that reported for CBD, supporting EPM301 as a distinct pharmacological entity rather than simply a CBD metabolite analogue. Following oral administration in rat and minipig, unchanged EPM301 was the predominant circulating component, with no significant accumulation observed for most metabolites following repeat dosing. Together, these findings provide important insight into the disposition of EPM301 and support the continued development of EPM301 as a potential therapeutic for Prader-Willi syndrome (PWS).

References: 1.Ben-Cnaan E., et al. *Int. J. Mol. Sci.* 2022;23(10):5610-5626. 2. Data on File. 3. FDA Centre for Drug Evaluation, Non-Clinical Review, 2018.

