Simultaneous Determination of Paracetamol and Orphenadrine Citrate in Rat Plasma using LC–MS/MS Spectrometry Method: Pharmacokinetics and Drug-Drug Interaction Profile Studies

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Abstract

Herein, it is the first developed and fully validated LC-MS/MS method for simultaneous determination of paracetamol (PAR) and orphenadrine citrate (ORP) in rat plasma after oral administration in respect of therapeutic drug monitoring, bioequivalence, pharmacokinetic (PK) and drug-drug interaction (DDI) studies. PAR and ORP were extracted from plasma sample using ACN-induced protein precipitation method. The separation procedure was carried out on an Agilent Eclipse Plus ODS (4.60 x 100 mm, 3.50 µm) column using water-enriched gradient mobile phase consisting of 6 mM ammonium formate plus 0.1% formic acid and ACN at a flow rate of 0.5 mL min^{-1}. The mass spectrometry parameters were optimized and multiple reaction monitoring (MRM) in positive ion mode of two transitions was utilized for quantification of precursor to production at *m/z* 152 → 110 & 65 for PAR as $[M+2H]^{2+}$, 270.3 → 181 for ORP as $[M + H]^{+}$. According to US-FDA bioanalytical requirements, the proposed method was verified in terms of linearity, selectivity, recovery, accuracy, precision, matrix effects, and stability. The method was linear in the range of 1-5000 and 1-500 ng mL^{-1} with detection limits (S/N of 3) of 0.12 and 0.07 ng mL^{-1} for PAR and ORP, respectively. The selectivity and high sensitivity of the method succeeded in the study of PK parameters and DDI between PAR and ORP after oral dose administration. The synergistic action between the two drugs was confirmed by studying variable parameters like K*a*, T_{max}, C_{max}, AUC_{0-24}, MRT, and Cl/F. PAR affects the absorption of ORP and decreases its C_{max} but increases its T_{max} in accordance with elevating AUC_{0-24} and reducing Cl/F for both drugs. The current PK research is a useful tool for determining the DDI of both medicines and might be used in therapeutic drug monitoring and bioequivalence studies.

1. Introduction

Paracetamol (PAR); N-(4-Hydroxyphenyl)acetamide (Fig. 1) is a painkilling and antipyretic drug worldwide [1]. PAR can act both centrally and peripherally when treating non-inflammatory conditions [2]. Orphenadrine citrate (ORP); (±)-N,N-Dimethyl-2-[(o-methyl-a-phenylbenzyl)oxy]ethylamine citrate (Fig. 1) is a skeletal muscle relaxant that can act centrally by depressing a specific neurons in the nervous system so that impulses of the somatic nerves can't be generated [2]. ORP is a chemical analogue of diphenhydramine and then can be used in the management of the neuroleptic syndrome and Parkinson disease [3]. The commercial combination of a pain reliever and a skeletal muscle relaxant is extensively utilized, and it outperforms single medicines in terms of prolonging and increasing the antinociceptive impact of PAR when combined with ORP [2].

The literature demonstrated that several methods have been carried out for the analysis of PAR & ORP in their pharmaceutical formulation or biological fluids. PAR & ORP were determined in their pharmaceutical formulation by spectrophotometric methods [2, 4–14], HPLC methods [15–17], TLC and microemulsion HPLC method [18], square wave voltammetric method [19] and capillary electrophoresis method [20]. Several methods have been reported for determination of PAR alone in biological matrices including spectrophotometric methods [21–24], HPLC methods [25–29], HPTLC method [30], GC method [31] and

voltametric methods [32–34]. Only chromatographic methods have been reported for determination of ORP alone in biological matrices including HPLC method [35] and GC methods [36–38]. However, only one method has been reported for simultaneous determination of PAR & ORP in spiked human serum by using HPLC method [39] but authors didn't study the pharmacokinetic (PK) profile of both drugs.

PK study plays an important role in drug discovery and development. It also enables a better connection and correlation between its dosing regimen and its exposure and responses. To the best of our knowledge, it is the first research paper that deals with PK and DDI between PAR and ORP. As such, the purpose of this study is to develop a simple, sensitive and reliable LC–MS/MS method for simultaneous determination of PAR and ORP in rat plasma, to better understand the PK profile of this synergistic combination which is commercially used all over the world.

2. Experimental

2.1. Chemicals and Reagents

All the Chemicals and reagents are of analytical grade. PAR & ORP were obtained as a gift from Egyptian International Pharmaceutical Industries Co. (EIPICO), Egypt. Acetonitrile (ACN) & methanol are HPLC grade and were obtained from Germany ((Riedel-de Haën Laboratory Chemicals, Selzer). Analytical grade ammonium formate and formic acid were bought from Germany (Merck company in Darmstadt, Germany). All stock, working and buffer solutions which are used for the mobile phase (aqueous solutions) are prepared by Milli-Q grade water (Millipore, Milford, MA, USA).

2.2. Chromatographic separation and MS/MS conditions

An Agilent 1260 series LC system (Agilent Technologies, Waldbronn, Germany) coupled with degasser (G4212B), Thermostat (G1330B), Quaternary pump (G1311B/C) along with auto-sampler (G1329B) was used to inject 10 µL aliquots of the used samples on an Agilent Eclipse Plus ODS (4.60 x 100 mm, 3.50 µm) column (Agilent Technologies, USA), which was kept at room temperature (25 ± 2°C) [40]. A gradient elution was carried out by altering the proportion of mixture A (6 mM ammonium with 0.1% formic acid buffer) and mixture B (ACN) as follow: 0–6.50 min (90% A - 10% B), at 6.5 min (60% A - 40% B), at 8 min (10% A - 90% B), at 10 min (0% A - 100% B), at 12 min (20% A - 80% B), and at 14 min (98% A - 2% B). The gradient mobile phase was delivered at a flow rate of 0.50 mL/min into the Electrospray ionisation chamber of mass spectrometer. PAR and ORP were identified using a triple–quadruple LC/tandem mass spectrometric detection system (Agilent 6460 QQQ LCMS) with a positive ionization electrospray ionization (ESI) interface. With a dwell time of 200 ns, the multiple reaction monitoring (MRM) transitions of m/z 152 → 110 & 65 for PAR and 270.3→ 181 for ORP were employed (Fig 2). At a flow rate of 600 Lh-1, nitrogen was used as a desolvating gas. The desolvation line temperature was 400 degrees Celsius, whereas the source temperature was 300 degrees Celsius, and the nebulizer pressure was 45 pounds per square inch. The collision gas flow, which was Argon, was 11 mL.min-1, with a capillary voltage of 4 kV. For PAR, the cone voltage and collision energy were set at 113 V and 39 eV, respectively, while for ORP,

they were set at 181V and 17 eV. Quantitative Analysis B.07.00 (the Mass Hunter software) was utilized to manage the UPLC–MS/MS system, and the Target Mass Hunter program was used for data collecting and processing [40].

2.3. Preparation of stock solution

PAR and ORP stock solutions (100 g mL^{-1}) were made by dissolving accurately weighed amounts of their respective standards in methanol and then using these solutions to make the calibration curve and quality control sample (QC).

2.4. Preparation of Calibration standards, QC samples

The PAR and ORP stock solutions were then diluted with ammonium formate-ACN (90:10, v/v) to make the calibration standards and QC sample working solutions. Plasma calibration standards (eight concentration levels) were prepared by dissolving in mobile phase and then spiking in rat blank plasma to reach final concentration levels of 1, 10, 50, 100, 500, 1000, 2500, 5000 ng mL^{-1} for PAR and 1, 5, 10, 20, 50, 100, 200, 500 ng mL^{-1} for ORP. In the same way, three QC samples were made at 10, 500, 2500 ng mL^{-1} for PAR and 10, 100, 500 ng mL^{-1} for ORP and treated as LQC, MQC, and HQC by dissolving in mobile phase or spiking in the blank plasma. Both plasma standards and QC samples were stored at -80 degrees Celsius until validation and/or optimization. The mobile phase samples could be stored at 4℃ for a month without changing.

2.5. Plasma sample preparation

Before preparation, plasma samples (calibration curve standards, QCs) were thawed at room temperature and vortexed for 30 seconds. One hundred microliters of plasma (QCs, blanks, and calibration standards) were added to a 1 mL Eppendorf tube, vortexed for 30 seconds, and deproteinized with 290 µL of ACN. At room temperature, the samples were centrifuged for 10 minutes at 10,000 g. The supernatant was separated and transferred to a new tube, which was maintained in the refrigerator until analysis, where 10 µL was injected into the LC-MS/MS.

2.6. Application to PK and DDI study in rats

The Suez Canal University Faculty of Pharmacy's Research Ethics Committee approved all the study's experimental protocols (Approved no. 201808RH1). All methods were carried out in accordance with the ARRIVE guidelines. Male Wistar Albino rats, 8 weeks old and weighing 200–250 g, were randomly placed into three groups, each with three rats. After an overnight fast, rats in Group I were given ORP (50 mg/Kg); rats in Group II were given PAR (450 mg/Kg); and rats in Group III were given ORP (50 mg/Kg) + PAR (450 mg/Kg) orally. After 0.5, 1, 2, 3, 4, 6, 8, 12, 24 hours, blood samples (each 300 µL) were obtained via the femoral artery into EDTA containers. Following blood collection, the rats were given an identical volume of saline in each case. The blood samples were centrifuged for 10 minutes at 10,000 g. The plasma layer was removed and transported to clean tubes, where it was kept until analysis at -80°C. To examine

method development and validation, blank plasma was taken from a rat that had not been given any drugs. The non-compartmental pharmacokinetic analysis was carried out using the PK Solver tool, which is a free add-in for Microsoft Excel. The linear trapezoidal approach was used to compute the area under the plasma concentration-time curve (AUC0-t). The experimental data was used to compute C_{max} (peak plasma concentration) and T_{max} (time necessary to achieve peak plasma concentration). Regression analysis was used to get K_{el} (the elimination rate constant) from the slope of the best fit line, and $T_{1/2}$ (the drug's half-life) was calculated using 0.693/ K_{el}. The PK Solver program was used to compute MRT (mean residence time), CL/F (total clearance of the drug from plasma), and Vd/F (volume of distribution) [40].

2.7. Method Validation

The following parameters were used to validate the method: selectivity and specificity were determined by comparing the chromatograms of blank rat plasma, plasma spiked with target analytes, and plasma samples collected from rats given PAR and ORP. LOD (limit of detection) and LOQ (limit of quantitation), which are concentrations with a signal-to-noise (S/N) ratio of at least 3.3 and 10, respectively, have been used to define sensitivity[40]. A least squares regression line was used to assess linearity, which was reported as r (correlation coefficient). On three distinct days, the linearity of the analytes was resolved by at least 8 concentration levels other than the blank [40]. The effect of plasma constituents on analyte ionization was assessed by comparing the responses of post-extracted plasma standard QC samples to the responses of analytes from neat samples at equivalent concentrations. Three QC samples were produced at low, middle, and high concentrations of 10, 500, 2500 ng mL^{-1} for PAR and 10, 100, 500 ng mL^{-1} for ORP, respectively, to assess intra- and inter-day precision and accuracy. The intraday precision was determined by calculating the percent RSD for the evaluation of QC samples in triplicates, whereas the interday precision was determined by examining QC samples on three different days. The relative error percentage ((measured conc - true conc / true conc) * 100) was obtained by comparing the averaged measurements to the nominal values. The peak area ratios of analytes in rat plasma at QC concentrations were compared to those in the mobile phase at corresponding concentrations and represented in percentages to demonstrate the recovery of PAR and ORP. The stability of the target analytes in rat plasma was examined for short periods of time (up to 24 hours) at room temperature (RT) and at 4°C, as well as for extended periods of time (up to two weeks) at -80 °C and after three freeze-thaw cycles [40].

3. Results And Discussion

3.1. Optimization of chromatographic conditions

The simultaneous chromatographic separation of ORP and PAR was complicated because the two drugs show a different nature. PAR has a slight lipophilicity (Log *P* = 0.31), whereas ORP is highly lipophilic with Log *P* = 3.50. Therefore, the quantitative determination of the two analytes in their combined mixture

either in dosage form or biological fluids is a continuous challenge. In the current study, various reversed phase columns were tried such as C_{18}, C_8, and CN. The reasonable retention of the target analytes has been accomplished on Agilent Eclipse Plus ODS (4.60 x 100 mm, 3.50 µm) column of C_{18} category. Aside from that, the mobile phase composition plays a critical role in chromatographic separation. Gradient elution was chosen for best separation because isocratic elution with variable quantities of buffer and organic modifier failed to allow effective separation of the two analytes, especially given the distinct nature of PAR and ORP. As an organic modifier, methanol and ACN were investigated; ACN was chosen because it provided appropriate analytes response and peak shape. In order to achieve the appropriate resolution and retention, the pH of the mobile phase played a substantial and crucial part in the separation process. Sharp peaks were achieved using a 6 mM ammonium formate buffer. Using ammonium formate-formic acid (pH 4.50) /ACN at gradient elution, good resolution and retention time with peak shape and analytes response were achieved, as detailed in the experimental section. In a 12-minute playtime, the retention time for PAR and ORP was 3.29 (Fig. 3) and 10.79, respectively (Fig. 4).

3.2. Optimization of mass spectroscopy condition

Using ESI sources in positive ion modes, 200 ng/mL solutions of the target analytes were directly injected to catch the protonated precursor ion and the most stable and intense product ion in a mass range of 20–200 amu for PAR and 100–500 amu for ORP. The Q1 MS exhibited predominant protonated precursor ions at *m/z* 152 and 270.3 for PAR and ORP, respectively. Most prominent and stable product ions for quantification of PAR and ORP were found at *m/z* 110 and 65 for PAR and at m/z 181 for ORP. The m/z 110 and 181 fragment ions for PAR and ORP, respectively, might be attributed to 4-aminophenol and N-methyl-2-((2-methylbenzyl) oxy) ethan-1-amine substructures produced from their respective precursor ions (Fig. 2).

3.3. Sample clean up and analytes extraction

Separation of interfering endogenous compounds from rat plasma samples was accomplished using SPE (solid phase extraction), LLE (liquid liquid extraction), and PP (protein precipitation). PAR recovery was poor in LLE in basic circumstances utilizing n-hexane, tert-butyl ether, and ethyl acetate as extraction solvents, although ORP recovery was promising (75 to 90%). Due to the differing nature of target analytes, the second attempt for analytes extraction was SPE using hydrophilic-lipophilic balance (HLB) cartilage. Despite the HLB cartilage attaining the required recovery, the significant matrix effect prohibited SPE from completing the experiment. For extracting PAR from biological fluids, the PP approach with ACN, ACN-formic acid, and/or methanol is extensively employed. The target analyte was successfully recovered using the PP approach with ACN as a precipitant in this study. The use of a three-fold amount of ACN to apply and precipitate low plasma volume was beneficial in reducing the matrix effect, preventing column damage, and increasing column lifetime.

3.4. Method validation

Based on the U.S. Guidance of Industry on Bioanalytical Method Validation, the current work has been validated under the parameters indicated in the Experimental Procedures section [41]. By graphing the

intensity of relative ions vs the concentration of the target analyte under ideal detection and separation circumstances, a linear connection was discovered. For PAR and ORP, the calibration curves constructed using analyte peak area versus concentration were linear in the ranges of 1-5000 ng mL^{-1} and 1-500 ng mL^{-1}, respectively, with excellent correlation coefficients (r) > 0.9997. The calibration curve standards' accuracy (%RE) and precision (%RSD) ranged from 92.70 to 101.10% and 1.10 to 3.20 for PAR and 96.30 to 100.20% and 1.35 to 3.94 for ORP. For PAR and ORP, the limit of detection (S/N of 3) was 0.12 and 0.07 ng mL^{-1}, respectively, with an RSD of less than 20%. Across three QC levels, the recovery of target analytes was in the range of 89.30 to 100.40% for PAR and 91.10 to 100.20% for ORP under optimal extraction conditions (Table 1). Peak response of post-extraction spiking / peak response of neat samples in mobile phase ranged from 0.83–1.30 and 0.79–1.40 for PAR and ORP, respectively. Method precision and accuracy, both between days and within day, was evaluated at different i.e. low, middle and high concentrations of PAR and ORP, as shown in Table 1. The accuracy calculated by relative standard error RE% ((Found Concentration-Add concentration/Add concentration) *100) of the proposed method either within day or between days was ranged between − 10.70 to 0.40% and − 8.90 to -0.19% for PAR and ORP, respectively. As shown in Table 1 for three QC samples, our suggested approach has excellent repeatability as measured by RSD percent (relative standard deviation). The investigated samples were processed quickly, ensuring the needed accuracy and precision. The method's selectivity was further tested using chromatograms from six different kinds of blank plasma[40]. Endogenous biological substances did not interfere with the retention duration of analytes in typical MRM chromatograms of extracted blank plasma and samples spiked with PAR and ORP. The suggested method outperforms prior methods for determining ORP or PAR in plasma in terms of sensitivity, simplicity, plasma volume, and sample processing, as well as its novelty in determining both analytes simultaneously utilizing the LC/MS methodology.

Table 1
Intraday and Interday Precision and Accuracy of ORP and PAR in Rat Plasma

	Measured Conc	Intraday		Recovery %	Measured Conc	Interday		Recovery %
	ng mL^{-1} ± SD	RSD%	RE%		ng mL^{-1} ± SD	RSD%	RE%	
ORP								
10	9.23 ± 0.88	9.53	-7.7	92.3	9.11 ± 1.03	11.3	-8.9	91.1
100	100.2 ± 2.13	2.13	-0.19	100.2	99.5 ± 1.65	1.66	-0.5	99.5
500	498.6 ± 2.22	0.45	-0.28	99.7	495.3 ± 2.17	0.44	-0.94	99.06
PAR								
10	8.93 ± 0.91	10.2	-10.7	89.3	9.12 ± 1.03	11.2	-8.8	91.2
500	490.2 ± 2.43	0.49	-1.96	98.04	493.3 ± 2.41	0.48	-1.34	98.66
2500	2510 ± 2.16	0.08	0.4	100.4	2492.1 ± 3.04	0.12	-0.316	99.68

3.5. Stability

The proposed method was effectively used to test the analytes' stability in the presence of plasma components. PAR and ORP stability were tested in the lab under a variety of settings, including short-term stability at room temperature and in the refrigerator for 24 hours, stability to three thaw-freeze cycles, and long-term stability by storing them at 80°C for two weeks [40]. The RSD percent values for the QC samples ranged from 0.08 to 10.20% for PAR and 0.24 to 9.53% for ORP under ideal experimental conditions (Table 2). In the presence of solvents and biological fluids, the target analytes exhibited reasonable stability. Working and stock solutions may be held at room temperature for a short period of time without degrading, while long-term stability was established at -80 ℃ for 15 days. PAR and ORP can also be preserved for a long period in biological fluids.

Table 2
Stability Data of ORP and PAR in Rat Plasma

	ORP			PAR		
Storage Condition	Nominal Conc	Found Conc	RSD%	Nominal Conc	Found Conc	RSD%
	ng mL^{-1}	ng mL^{-1} ± SD		ng mL^{-1}	ng mL^{-1} ± SD	
4°C temperature for 1 h	10	9.23 ± 0.88	9.53	10	8.93 ± 0.91	10.2
	100	100.2 ± 2.13	2.13	500	490.2 ± 2.43	0.49
	500	498.6 ± 2.22	0.45	2500	2510 ± 2.16	0.08
Refrigerator (4°C) for 24 h	10	8.96 ± 1.21	13.5	10	9.57 ± 0.87	9
	100	98.2 ± 1.13	1.15	500	491.2 ± 2.06	0.42
	500	489.2 ± 2.18	0.44	2500	2489.3 ± 3.1	0.12
−80°C temperature for 15 days	10	9.43 ± 0.91	9.6	10	9.14 ± 1.09	11.9
	100	98.9 ± 1.14	1.15	500	496.7 ± 1.8	0.36
	500	500.1 ± 1.22	0.24	2500	2500.2 ± 2.44	0.09
Three freeze-thaw cycles (each at − 80°C)	10	9.17 ± 1.08	11.8	10	9.2 ± 0.74	0.8
	100	99.12 ± 3.2	3.22	500	488.3 ± 1.76	0.36
	500	491.3 ± 2.91	0.59	2500	2489.4 ± 2.5	0.1

3.6. PK and DDI Studies in Rats

After oral dosage administration of 450 and 50 mg kg^{-1} of PAR and ORP, respectively, either alone or in mixed doses, the current completely validated LC-MS/MS was successful in measuring the PK parameters of PAR and ORP in rat plasma. Table 3 summarized the key PK parameters of PAR and ORP determined using PK Solver, and Fig. 5 depicted the mean plasma concentration-time curves. With a high

area under the curve (AUC_{0-24}) of 7063.96 ng $mL^{-1}h^{-1}$, a peak plasma concentration (Cmax) of 3010.65 ng mL^{-1} was reached. PAR had a short terminal half-life ($t_{1/2}$) of 1.47 h and a mean residence duration of 2.10 h, owing to a wide distribution volume of 13.40 L and a high plasma clearance of 6.30 Lh^{-1}. ORP, on the other hand, had a low AUC_{0-24} of 413.16 ng $mL^{-1}h^{-1}$ and a Cmax of 449.90 ng mL^{-1}, both of which were achieved quickly (Tmax = 1.00 h). This is due to its high volume of distribution (34.20 L) and high plasma clearance (11.50 Lh^{-1}) causing it to accumulate in the tissues.

Table 3
Pharmacokinetic Parameters of ORP and PAR in Rat Plasma after Oral Administration of Single Dose and Combined Doses

Parameter	Unit	ORP (50 mg Kg^{-1})		PAR (450 mg Kg^{-1})	
		Alone	With PAR (450 mg Kg^{-1})	Alone	With ORP (50 mg Kg^{-1})
Ka	h^{-1}	4.9 ± 0.67	2.76 ± 1.5	3.2 ± 0.72	4 ± 1.3
λ_z	h^{-1}	0.336 ± 0.12	0.28 ± 0.09	0.47 ± 0.12	0.52 ± 0.21
$t_{1/2}$	h	2.05 ± 0.81	2.46 ± 1.45	1.47 ± 0.88	1.3 ± 0.91
T_{max}	h	1 ± 0.23	3 ± 1.66	1 ± 1.5	1 ± 0.36
C_{max}	ng/ml	449.9 ± 35.6	366.6 ± 44.5	3010.65 ± 534	4767.8 ± 296
AUC_{0-t}	ng/ml*h	413.16 ± 54.6	1335.5 ± 129.4	7063.96 ± 482	11517.5 ± 254
AUC_{0-inf}	ng/ml*h	433.6 ± 63.2	1451.1 ± 18.5	7103.65 ± 163	11556.38 ± 345
MRT	h	2.1 ± 1.34	5.28 ± 1.23	2.1 ± 0.47	2.5 ± 0.17
K_{el}	h^{-1}	0.99 ± 0.27	0.55 ± 0.33	0.65 ± 0.13	0.83 ± 0.04
V/F	L	34.2 ± 11.4	12.2 ± 4.1	13.4 ± 2.3	7.4 ± 1.5
Cl/F	Lh^{-1}	11.5 ± 2.42	34.4 ± 14.1	6.3 ± 1.15	3.8 ± 0.46

The addition of ORP, which is a skeletal muscle relaxant, could enhance the effect of PAR and then the combination could be used in different diseases [14]. This combination is well studied pharmacologically but there is no reported method that deals with that kind of PK and DDI studies between both drugs. PAR is mainly metabolized through conjugation with glucuronate and sulfate moieties by Phase II metabolism or through minor pathway by the cytochrome P450 to produce a toxic metabolite, NAPQI. On the other

hand, ORP is also metoabolized by hepatic pathway but through N- and N,N- demethylation, therefore, there is evidence that the metabolism has a limited role in DDI between PAR and ORP. However, as shown in Table 3, the clinical findings here confirm that the co-administration of PAR and ORP could influence the PK profiles of each other in terms of K*a*, T_{max}, C_{max}, AUC_{0-24}, MRT, and Cl/F. There is an evident interaction between PAR and ORP at site of absorption where K*a* of PAR increased by 25% while that of ORP decreased by around 56% and this can explain the concomitant increase in C_{max} value for PAR and vice versa in case of ORP. Additionally, T_{max} of ORP increased twice from 1 to 3 h and this indicates that ORP was more influenced by the co-administration process than PAR. AUC_{0-24} of both PAR and ORP was also increased by 63% and 235%, respectively, when the two drugs were co-administered, and this leads to an increase in the MRT specially in case of ORP (151%). Moreover, Cl/F for both drugs was greatly decreased and this indicates that there is pharmacokinetic interaction between the two drugs which is mainly synergistic.

4. Conclusion

To shed light on the PK explanation of the risk/benefit effects of PAR and ORP combined in pain management, a unique LC-MS/MS technique was developed. The proposed method can be used to investigate the PK characteristics of individual drugs or combinations of drugs. The new method, which is the first of its type, validates DDI between PAR and ORP when they are co-administered at the same time, which is a significant benefit for bioequivalence and dose monitoring in the treatment of inflammation.

Declarations

Ethics approval and consent to participate

The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

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Availability of data and materials

The datasets used and analyzed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Contributions

Mohamed Saleh Elgawish, Amr A. Mattar, and Mahmoud M. Sebaiy designed and wrote the research work, while Sobhy M. El-Adl revised the manuscript and supervised the research. All authors read and approved the final manuscript.

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Figures

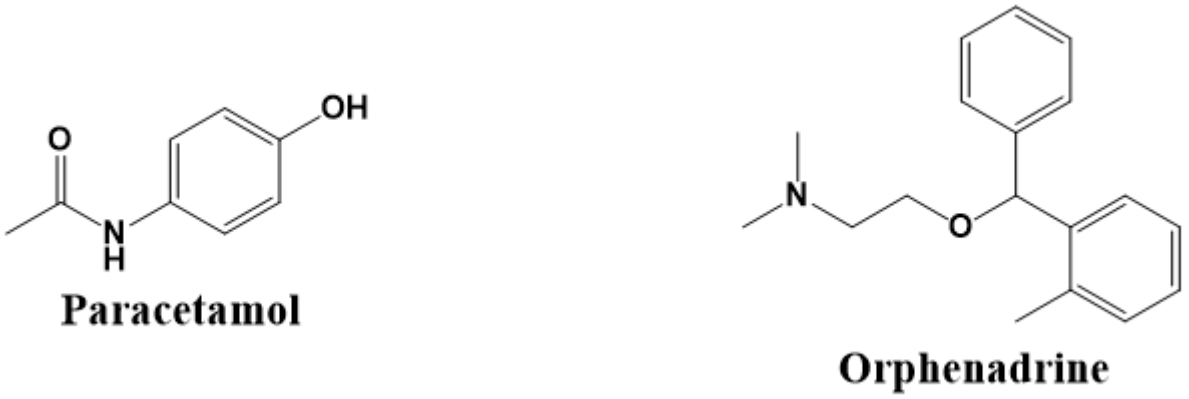
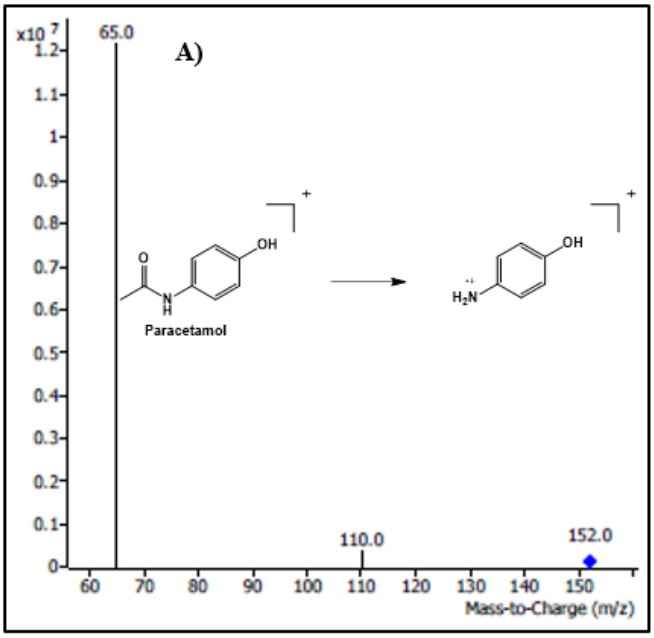


Figure 1

Chemical structures of PAR and ORP



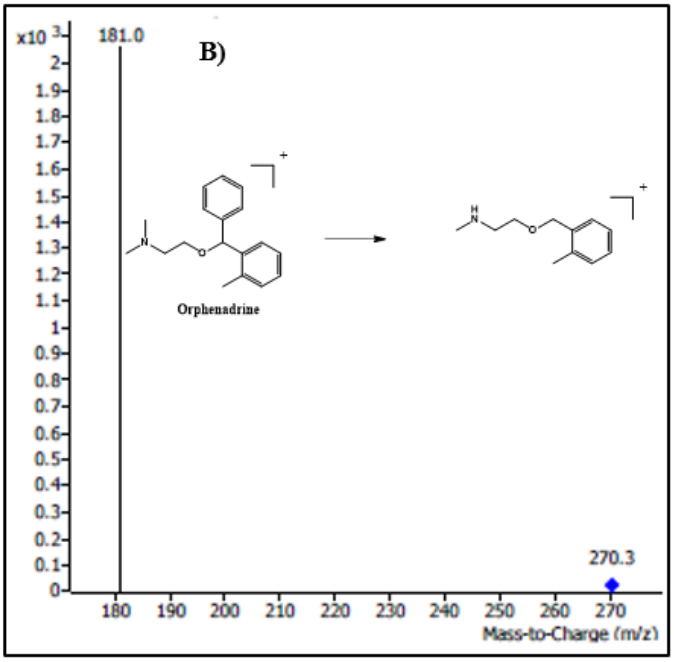


Figure 2

Precursor ion spectra in positive ion mode (A) of PAR and (B) of ORP.

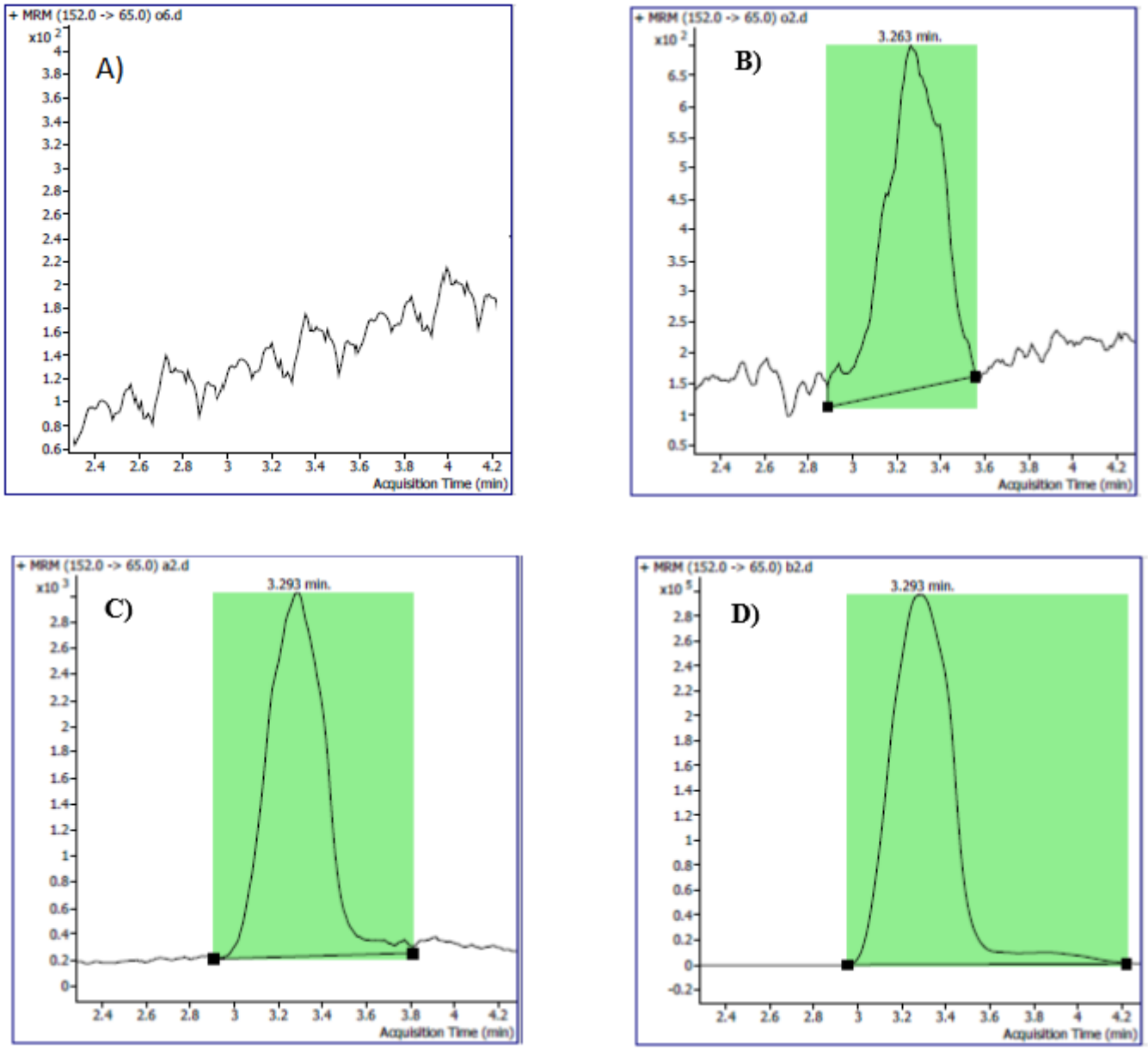


Figure 3

Typical MRM chromatograms of PAR in rat plasma: (A) blank sample; (B) blank plasma spiked with LLOQ; (C) plasma sample from rat 0.5 h after oral administration of PAR; (D) plasma sample from rat 1 h after oral administration of PAR.

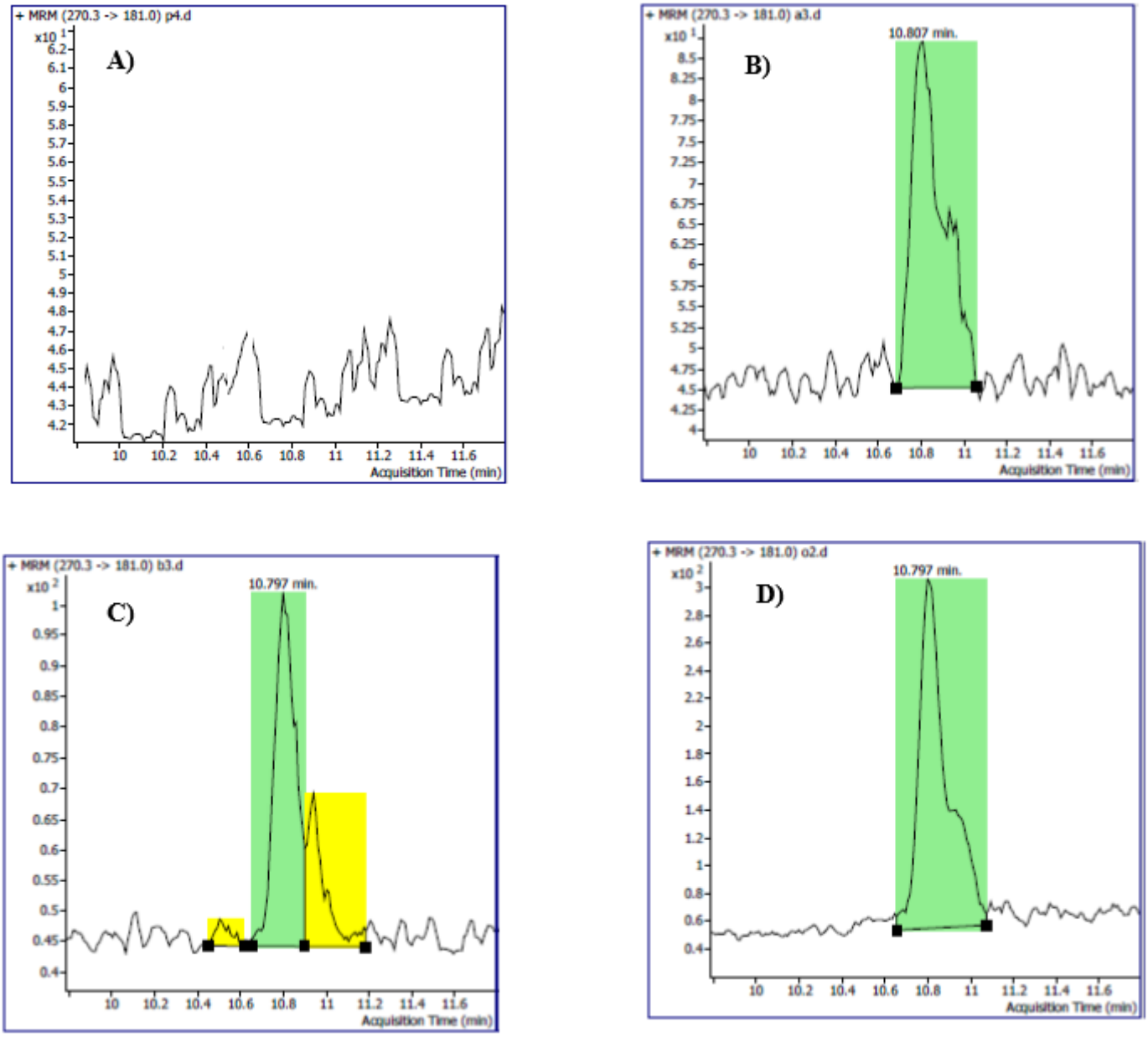
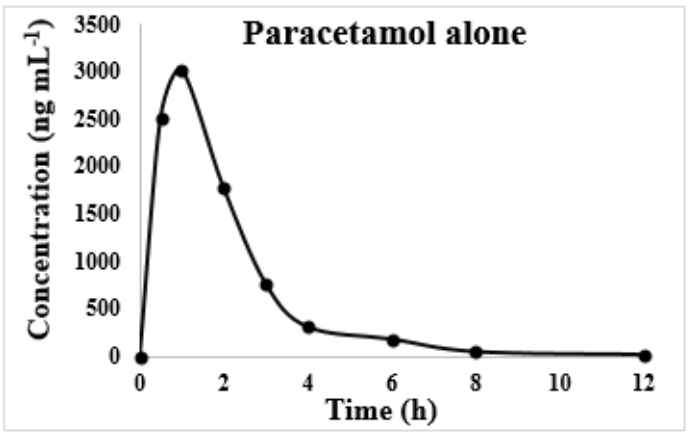
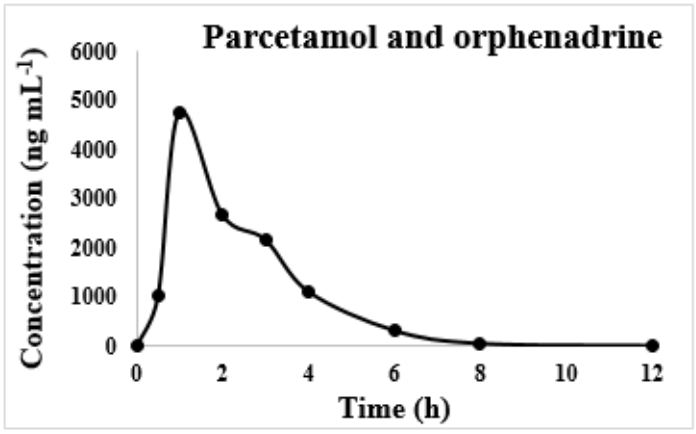
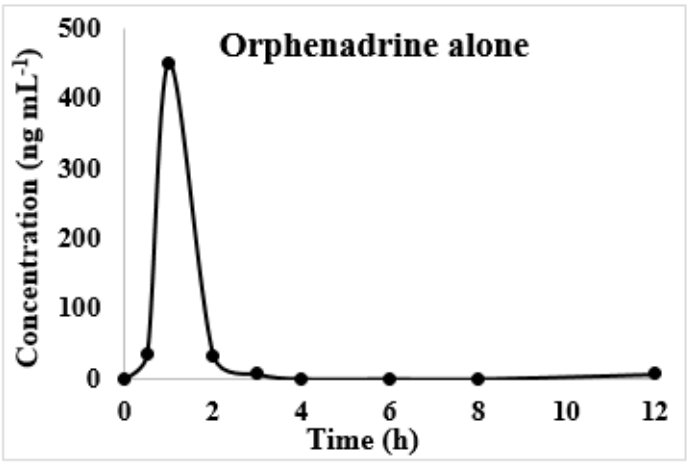


Figure 4

Typical MRM chromatograms of ORP in rat plasma: (A) blank sample; (B) blank plasma spiked with LLOQ; (C) plasma sample from rat 0.5 h after oral administration of PAR; (D) plasma sample from rat 1 h after oral administration of PAR.







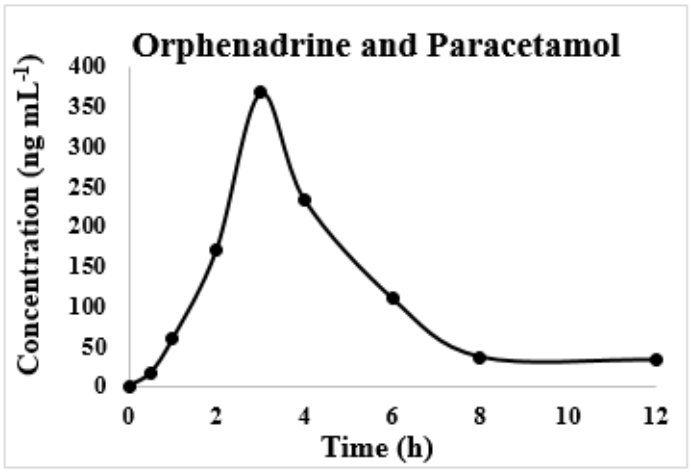


Figure 5

Mean ± SE plasma concentration–time curves of PAR (450 mg kg^{-1}) and ORP (50 mg kg^{-1}) after a single and combined dose administration.