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ARTICLES

Development of an UHPLC-UV Method for Quantification of Three Phenyl 4-(2-oxo-3-alkylimidazolidin-1-yl)benzenesulfonates in CD-1® IGS Female Mouse Plasma

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Abstract—Phenyl 4-(2-oxo-3-alkylimidazolidin-1-yl)benzenesulfonates (**PAIB-SO**s) are new antimitotic prodrugs bioactivated by the enzyme CYP1A1 into phenyl 4-(2-oxo-3-imidazolidin-1-yl)benzenesulfonates (**PIB-SO**s) that are potently (nM) and selectively inhibiting the growth of CYP1A1-expressing breast cancer cells. The screening program identified three PAIB-SOs designated as CEU-835, CEU-934, and CEU-938 for further pharmacokinetic studies. A novel method for their quantification in CD-1® IGS female mouse plasma using a Waters UHPLC ACQUITY Arc system was developed and validated. Their analytical measurements ranged from 7.3 to 1484 ng/mL. They exhibited linearity between 7.3 and 1484 ng/mL. They also showed good resolutions (2.21–4.99) and good theoretical plate numbers (14707–70580). Moreover, they were robust at wavelengths between 273 and 310 nm and sensitive from 2 to 62 AU/ng mL^{-1}. This new method is indispensable to assess PAIB-SOs half-life in mouse plasma and for the quantification of PAIB-SOs in other biological matrices.

Keywords: detection and validation method, quantification method, CYP1A1-activated antimicrotubule prodrugs, phenyl 4-(2-oxo-3-alkylimidazolidin-1-yl)benzenesulfonates, PAIB-SOs, CEU-835, CEU-934, CEU-938

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Breast cancer is a major health problem, accounting for the first neoplasia-related cause of death worldwide [1]. Accordingly, there is a strong demand for the development of new breast cancer therapies. In this context, a new family of anticancer agents has been designated such as phenyl 4-(2-oxo-3-alkylimidazolidin-1-yl)benzenesulfonates also known as **PAIB-SO**s [2–5]. PAIB-SOs are prodrugs and, therefore, inactive in most normal and cancer cells. Nevertheless, they become highly cytocidal (nM) in human CYP1A1-expressing breast cancer cells. In addition, they are showing high selectivity ratios (2 to 3 logs) between cells expressing CYP1A1 and cells devoid of CYP1A1. PAIB-SOs undergo a bioactivation process via CYP1A1-mediated *N*-dealkylation, occurring on the N^3 atom of the 2-imidazolidone moiety (aromatic ring A). This produces potent antimitotics referred to as phenyl 4-(2-oxoimidazolidin-1-yl)benzenesulfonates (**PIB-SO**s) and exemplified in Fig. 1 by compounds CEU-722, CEU-733, and CEU-700. CEU-722, CEU-733, and CEU-700 bind to the colchicine-binding site of microtubules, leading to cytoskeleton disruption, arrest of the cell cycle progression in the G2/M phase, and ultimately to the cell death [6, 7]. PAIB-SOs represent a promising new class of highly potent anticancer prodrugs for the treatment of CYP1A1-expressing breast cancer tumors.

The screening program on the development of PAIB-SOs using several biofunctional assays led to the identification and selection of the three promising PAIB-SOs named 3-chlorophenyl 4-(2-oxo-3-pentylimidazolidin-1-yl)benzenesulfonate (CEU-835), 3-iodophenyl 4-(2-oxo-3-pentylimidazolidin-1-yl)benzenesulfonate (CEU-934), and 3,5-dichlorophenyl 4-(2-oxo-3-pentylimidazolidin-1-yl)benzene-

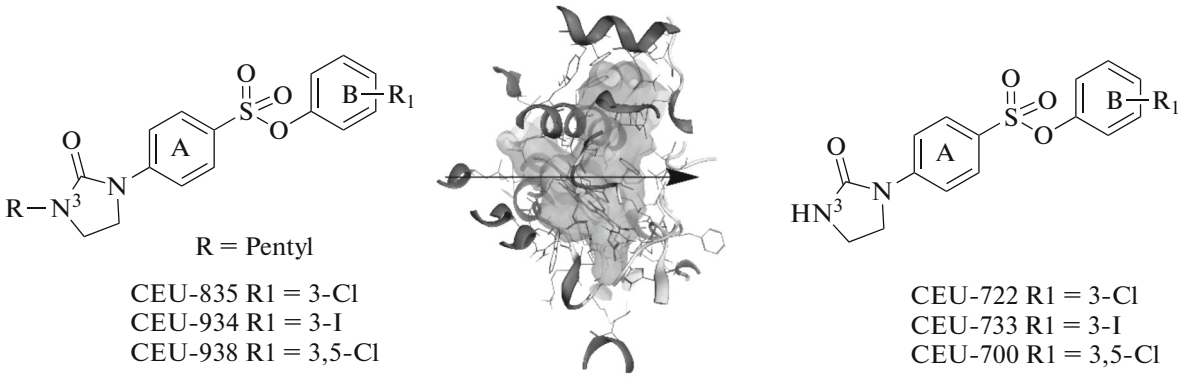


Fig. 1. Molecular structure and CYP1A1-mediated *N*-dealkylation of 4-(2-oxo-3-alkylimidazolidin-1-yl)benzenesulfonates (PAIB-SOs) into highly potent antimitotic phenyl 4-(2-oxoimidazolidin-1-yl)benzenesulfonates (PIB-SOs).

sulfonate (CEU-938) (Fig. 1), respectively, for further studies in animal models of cancer [5]. One of the most important steps before undertaking costly studies is the development and validation of methods for their quantification in animal models. Moreover, the biological matrix preferentially used to perform pharmacokinetic studies is plasma by blood collection [8, 9]. Therefore, the purpose of this study was to develop and validate new quantification methods of CEU-835, CEU-934, and CEU-938 in plasma using UHPLC-UV and plasma from CD-1® IGS female mice.

EXPERIMENTAL

Reagents. The following chemical reagents were used: Forane® (isoflurane, USP, Baxter Corporation, ON, Canada), dimethyl sulfoxide (purity ≥99.7%, Thermo Fisher Scientific, ON, Canada), tetraglycol (MQ200 grade, MilliporeSigma, ON, Canada), ethanol (ACS reagent grade, anhydrous, Thermo Fisher Scientific, ON, Canada), Tween® 80 (Proteomics grade, VWR International, ON, Canada), (±)-1-methoxy-2-propanol (purity ≥99.0%, VWR International, ON, Canada), D-glucose (Thermo Fisher Scientific, ON, Canada), sodium chloride (Thermo Fisher Scientific, ON, Canada), methanol (Optima™ for HPLC, purity ≥99.0%, Thermo Fisher Scientific, ON, Canada), and ultrapure water (PURELAB® flex, ELGA LabWater, Culligan, IL, USA).

Sample preparation. All blood samples were obtained from CD-1® IGS female mice (Charles River Laboratories, MA, USA). In vivo experiments in this study were approved by the Animal Care Committee of the Centre de recherche du CHU de Québec-Université Laval under the authorization numbers 18-014-1 (CPAC) and 19-019-1 (CPAUL-3). Briefly, 20 healthy mice were maintained in groups of five individuals per cage until their average weight reached 25 g. Then, a terminal cardiac puncture was performed under isoflurane anesthesia using a 25G needle and 1 mL syringes (BD Biosciences, ON, Canada) to collect the blood samples. Blood samples were transferred in potassium EDTA-containing microtubes (Microvette®, Sarstedt, QC, Canada), and plasma was separated from whole blood by centrifugation at 1×10^3 g at 4°C for 15 min. Plasma was stored at 2–8°C until experimental procedures.

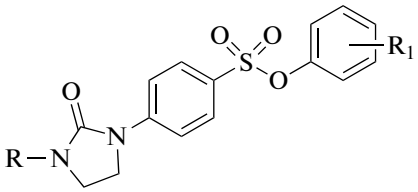
The validation samples were produced by ex vivo addition of solutions of PAIB-SO and internal standard (**IS**) in plasma. Briefly, CEU-835, CEU-934, or CEU-938 were dissolved at the concentrations described in Table 1 in a mixture of tetraglycol, ethanol, tween® 80, propylene glycol, glucose 10%, and NaCl 0.9% (15, 1.6, 13.6, 5.5, 21.8, and 42.5%, respectively) for CEU-835 and CEU-938 or a mixture of tetraglycol, ethanol, tween® 80, propylene glycol, glucose 10%, and NaCl 0.9% (15, 2.06, 17.65, 7.06, 28.23, and 30%, respectively) for compound CEU-934. Escalating concentrations of CEU-835, CEU-934, or CEU-938 were obtained by adding 2.5 μL of the above-described solutions to 35 μL of plasma to which 2.5 μL of an IS solution (200 μM in DMSO, 75.7 to 106.5×10^3 ng/mL) were added.

Equipment and analysis method. All UHPLC-UV analyses were performed using a UHPLC ACQUITY Arc system (Waters, ON, Canada) equipped with a 2998 UV/visible photodiode array detector. The samples were eluted using a mixture of methanol/water with a linear mobile phase gradient (1.0 mL/min) on a CORTECS C18+ silica-based reversed-phase column (90 Å, 3.0 × 50 mm, 2.7 μm) paired with a CORTECS C18+ VanGuard Cartridge (90 Å, 2.1 × 5 mm, 2.7 μm, Waters, ON, Canada). A gradient of a water/methanol mixture was used for all UHPLC-UV analyses at 60 : 40 (0.0–2.0 min), 60 : 40 to 35 : 65 (2.0–2.5 min), 35 : 65 to 0 : 100 (2.5–4.0 min), 0 : 100 (4.0–4.01 min), and 60 : 40 (4.01–5.5 min) at 1.0 mL/min. The water/methanol mixture was preferred over water/ethanol, water/acetone, and water/acetonitrile mixtures due to weaker solubility of our compounds in the other solvent mixtures assessed. The wavelength used for analysis was 280 nm with the

Table 1. Summary of the highest and lowest concentrations (ng/mL) used to obtain the straight-line equations for CEU-835, CEU-934, and CEU-938 in both the vehicle and plasma

Standard candidate	Vehicle		Plasma	
	lowest	highest	lowest	highest
CEU-835	21	1692	15	1220
CEU-934	5	3087	2	1484
CEU-938	4	3659	2	1319

Table 2. Internal standard (IS) candidates for the quantification and validation methods for CEU-835, CEU-934, and CEU-938 using UHPLC-UV



Internal standard candidate	Name	R	R_1
CEU-602 [7]	3,4,5-Trimethoxyphenyl 4-(2-oxoimidazolidin-1-yl)benzenesulfonate	H	3,4,5-OMe
CEU-699 [7]	3,5-Dimethoxyphenyl 4-(2-oxoimidazolidin-1-yl)benzenesulfonate	H	3,5-OMe
CEU-818 [2]	3,4,5-Trimethoxyphenyl 4-(3-butyl-2-oxoimidazolidin-1-yl)benzenesulfonate	*n*-Butyl	3,4,5-OMe
CEU-820 [2]	3,5-Dimethoxyphenyl 4-(3-butyl-2-oxoimidazolidin-1-yl)benzenesulfonate	*n*-Butyl	3,5-OMe
CEU-827 [4]	3,4,5-Trimethoxyphenyl 4-(3-(sec-butyl)-2-oxoimidazolidin-1-yl)benzenesulfonate	*sec*-Butyl	3,4,5-OMe
CEU-924 [4]	3,5-Dichlorophenyl 4-(3-isobutyl-2-oxoimidazolidin-1-yl)benzenesulfonate	Isobutyl	3,5-Cl
CEU-925 [4]	3,5-Dibromophenyl 4-(3-isobutyl-2-oxoimidazolidin-1-yl)benzenesulfonate	Isobutyl	3,5-Br
CEU-926 [4]	3,4,5-Trimethoxyphenyl 4-(3-isobutyl-2-oxoimidazolidin-1-yl)benzenesulfonate	Isobutyl	3,4,5-OMe

exception of the robustness test. The built-in tool in the Empower Acquity Arc Waters software called Waters Compare, allowing the overlay of chromatograms, was used to validate that peaks match with the identified compounds.

Internal standard selection. The molecules chosen as IS candidates (Table 2) were selected based on the similarity of their structures with CEU-835, CEU-934, and CEU-938. To that end, three parameters were considered: (1) the retention time (**RT**) of each IS candidate, (2) the RT delta (Δ) between IS and PAIB-SOs, and (3) the height peak ratio (Height $Ratio_{IS/PAIB\text{-}SO}$) between IS peak height ($Height_{IS}$) and selected PAIB-SO peak height ($Height_{PAIB\text{-}SO}$). The ratio of peak heights was evaluated using Eq. (1).

$$\text{Height Ratio}_{IS/PAIB-SO} = \frac{\text{Height}_{IS}}{\text{Height}_{PAIB-SO}}, \quad (1)$$

where $Height_{IS}$—peak height of the internal standard, $Height_{PAIB\text{-}SO}$—peak height of the tested PAIB-SO.

Sample reconstitution. The samples were pretreated by adding 500 μL of methanol to 40 μL of the plasma samples. The mixtures were then centrifuged for 5 min at 1.5×10^4 g. The supernatants were separated from the pellet and were then filtered using a solid phase extraction column (SPE, IRIS™ MCX, 60 mg/3 mL, 25–35 μm cartridges, Canadian Life Sciences, ON, Canada) preconditioned with 500 μL of methanol. The solutions were collected and 100 μL of DMSO were added to avoid total evaporation. The mixtures were evaporated to a residual volume of approximately 50 μL using a SpeedVac® Vacuum Concentrator (Thermo Fisher Scientific, MA, USA) for 1.5–2 h. The samples were reconstituted in a final mixture of 20% DMSO, 40% methanol, and 40% water and were analyzed by UHPLC-UV.

Method validation. Two matrices were used for the validation of CEU-835, CEU-934, and CEU-938 that are a pure solvent mixture containing DMSO, methanol, and water (20 : 40 : 40), and blood plasma, respectively. All the calculations that include arithmetic means used the arithmetic mean equation [10]. The calculations that need the standard deviation of the replicates were performed using the standard deviation of a population equation [11]. The straight-line equations were obtained by analyzing CEU-835,

CEU-934, and CEU-938 at different concentrations (Table 1). These ranges of concentrations were also used for the determination of the parameters for the validation, the detection limits, and the quantification limits. The instrumental detection limit (**IDL**) is defined as three times the standard deviation (**Sn**) of at least 10 replicates of PAIB-SO in the pure solvent matrix and was calculated using the IDL equation (Eq. (2)) [12].

$$\mathrm{IDL} = 3(\mathrm{Sn}). \quad (2)$$

The method detection limit (**MDL**) is defined as three times the standard deviation of at least 10 replicates of PAIB-SO in the sample matrix and was calculated using the MDL equation (Eq. (3)) [12].

$$\mathrm{MDL} = 3(\mathrm{Sn}). \quad (3)$$

The validity of the IDL and MDL was evaluated by calculating the R conformity value using the R equation (Eq. (4)).

$$\mathrm{R} = \frac{\bar{\mathrm{X}}}{\mathrm{IDL\ or\ MDL}} = \frac{\bar{\mathrm{X}}}{3(\mathrm{Sn})}. \quad (4)$$

The limit of quantification of the method (**LOQ**) is defined as 10 times the standard deviation of at least 10 replicates in sample matrix and was calculated using the LOQ equation (Eq. (5)) [12].

$$\mathrm{LOQ} = 10(\mathrm{Sn}). \quad (5)$$

The sensibility ($\boldsymbol{m_c}$) was calculated using the general slope equation considering the absorbance signal units (**SU**) of the UHPLC. The SU are compared to the concentration of PAIB-SOs, expressed as arbitrary UHPLC units per ng/mL (AU/ng mL^{-1}) (Eq. (6)).

$$m_c = \frac{(\mathrm{SU}_{c_2} - \mathrm{SU}_{c_1})}{c_2 - c_1}, \quad (6)$$

where SU_{c1}—signal units for the first concentration, SU_{c2}—signal units for the second concentration, c_1—first concentration, c_2—second concentration.

The robustness was evaluated using the resolution ($\boldsymbol{R_s}$) of the IS and PAIB-SO co-eluted in sample matrix at different wavelengths. The R_s was calculated using the R_s equation (Eq. (7)) [13].

$$R_s = \frac{(\mathrm{RT}_2 - \mathrm{RT}_1)}{\frac{1}{2}(\mathrm{WB}_1 - \mathrm{WB}_2)}, \quad (7)$$

where RT_1—retention time of the first peak, RT_2—retention time of the second peak, WB_1—base width of the first peak, WB_2—base width of the second peak.

Finally, the robustness of the method at different wavelengths was calculated by the theoretical plate number (N) also known as plate count. The N is a measure of the efficiency of the column defined by Eq. (8) [14].

$$N = 16\left(\frac{\mathrm{RT}}{\mathrm{WB}}\right)^2, \quad (8)$$

where RT—retention time of the peak, WB—base width of the peak.

Finally, all the errors are expressed as the standard error of the mean (**SEM**).

RESULTS AND DISCUSSION

CEU-926 was selected as the internal standard for the sample matrix analyses. As shown in Table 3, the RTs of most of the IS candidates are shorter than for CEU-835, CEU-934, and CEU-938. Several IS candidates were considered acceptable in the pure solvent matrix. However, several additional peaks were found in plasma from residual plasmatic proteins. In this context, CEU-926 was identified as a promising IS for the analyses of CEU-835, CEU-934, and CEU-938 since its RT (2.942 min) is shorter than the analyzed PAIB-SOs and it does not overlap with plasma-derived impurities. Moreover, it exhibits an RT difference (Δ) between the most promising PAIB-SOs ranging from 0.248 to 0.377 min. In addition, the ratio of height was evaluated using compound CEU-938 as a PAIB-SO model to determine their relative absorbance, which should be similar. The height ratio between CEU-926 and CEU-938 is 0.99, indicating very similar absorbance between both PAIB-SOs. CEU-926 was thus considered a suitable IS for validation of the method for CEU-835, CEU-934, and CEU-938 determination, and it was used for all subsequent quantification experiments.

The UHPLC-UV detection was validated for IDL, MDL, and LOQ, and the linearity of the method was established. At least 10 replicates of injection of each compound were analyzed for the validation process and for repeatability. The RT of both PAIB-SOs and IS were recorded and are listed in Table 4.

As shown in Fig. 2, the concentrations for the validation of the detection method for CEU-835, CEU-934, and CEU-938 were separated into a non-quantifiable range (0–IDL and IDL–MDL), a semi-quantifiable range (MDL–LOQ), and the analytical measurement range (LOQ–max solubility). The highest concentration that was evaluated does not correspond to the maximum soluble concentration. It corresponds to the highest concentration attainable to avoid precipitation of the tested compounds in the UHPLC, that may result in a blockage of the system.

Sample chromatograms analyzed with the UHPLC-UV method described in the equipment section for CEU-835, CEU-934, and CEU-938 for the highest concentration that was evaluated in plasma-derived treated samples, which corresponds to 2.8 μM, are presented in Fig. 3. The IDL and MDL are the lowest concentrations that can be detected in the solvent and the sample matrices, respectively, and that can be statistically distinguished with 99% confidence from method blank results. The IDL and MDL were 2.1 and 3.8 ng/mL for CEU-835, 1.1 and

Table 3. Retention times, retention time differences, and height ratios between the internal standard candidates (CEU-602, CEU-699, CEU-818, CEU-820, CEU-827, CEU-924, CEU-925, and CEU-926) and standard candidates (CEU-835, CEU-934, and CEU-938)

Standard and internal standard candidate	RT, min	$\Delta RT_{IS}-RT_{CEU-835}$	$\Delta RT_{IS}-RT_{CEU-934}$	$\Delta RT_{IS}-RT_{CEU-938}$	Height $ratio_{IS/CEU-938}$
CEU-835	3.190	N.A.	N.A.	N.A.	N.A.
CEU-934	3.215	N.A.	N.A.	N.A.	N.A.
CEU-938	3.319	N.A.	N.A.	N.A.	N.A.
CEU-602	1.822	1.368	1.393	1.497	0.44
CEU-699	2.287	0.903	0.928	1.032	0.46
CEU-818	2.971	0.219	0.244	0.348	1.09
CEU-820	3.082	0.108	0.133	0.237	0.88
CEU-827	2.901	0.289	0.314	0.418	0.97
CEU-924	3.256	−0.066	−0.041	0.063	1.42
CEU-925	3.287	−0.097	−0.072	0.032	0.79
CEU-926	2.942	0.248	0.273	0.377	0.99

N.A.—non-applicable.

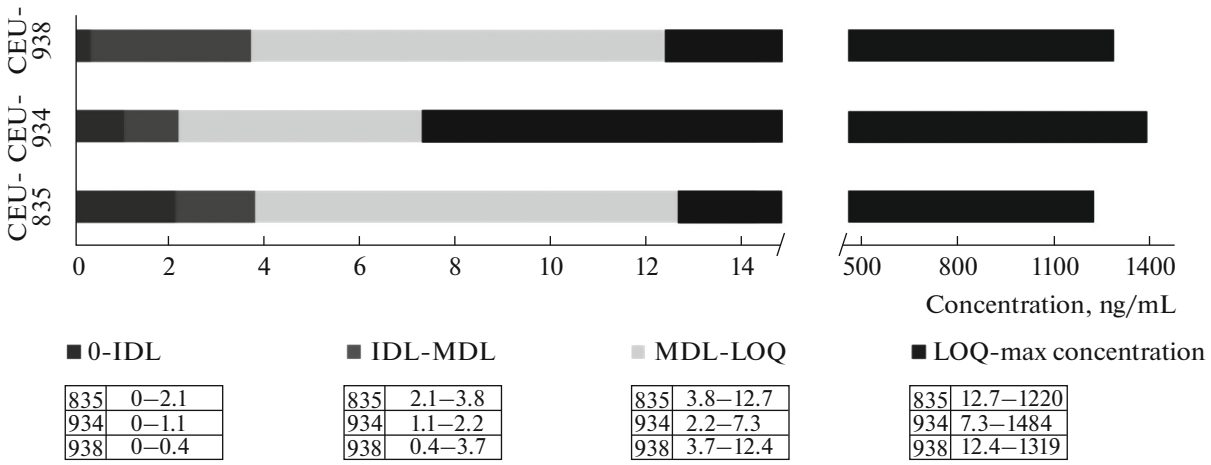
Table 4. Retention time (min) of CEU-835, CEU-934, CEU-938, and CEU-926 (IS)

Sample	CEU-835	IS	CEU-934	IS	CEU-938	IS
1	3.180	2.937	3.174	2.936	3.313	2.938
2	3.180	2.937	3.173	2.935	3.290	2.892
3	3.180	2.937	3.175	2.939	3.309	2.942
4	3.178	2.935	3.173	2.936	3.309	2.942
5	3.177	2.936	3.175	2.937	3.310	2.944
6	3.181	2.934	3.175	2.937	3.311	2.944
7	3.183	2.936	3.174	2.935	3.309	2.942
8	3.178	2.936	3.175	2.936	3.310	2.944
9	3.183	2.935	3.176	2.938	3.310	2.945
10	3.178	2.934	3.176	2.937	3.310	2.944
Mean ± SEM	3.180 ± 0.001	2.9357 ± 0.0004	3.1746 ± 0.0003	2.9366 ± 0.0004	3.308 ± 0.002	2.938 ± 0.005

2.2 ng/mL for CEU-934, and 0.4 and 3.7 ng/mL for CEU-938, respectively.

The ranges for the non-quantifiable zones indicate that obtained concentrations of a sample in this zone are not reliable, as the error is significant and strongly affects the outcome (below 3.8, 2.2, and 3.7 ng/mL for CEU-835, CEU-934, and CEU-938, respectively). The semi-quantifiable zones (IDL–MDL and MDL–LOQ) represent a quantification zone in which the error could potentially affect the outcome, and the results obtained in that range are not completely reliable (between 3.8 and 12.7, 2.2 and 7.3, and 3.7 and 12.4 ng/mL for CEU-835, CEU-934, and CEU-938, respectively). The obtained concentrations that are higher than the semi-quantifiable zone and lower than the maximum of linearity (or maximum of solubility) are reliable in terms of validity and are defined as the quantifiable zone. The quantifiable zone ranges from 12.7–1220, 7.3–1484, and 12.4–1319 ng/mL for CEU-835, CEU-934, and CEU-938, respectively.

The validation method must be verified for each compound using the R conformity value. The compliance of the validation of the methods is shown in Table 5 and was calculated using the R conformity value. The R value of a valid method must range from 4 to 10. The R values for IDL and MDL are 8.20 and 4.43 for CEU-835, 4.04 and 7.23 for CEU-934, and 4.97 and 4.18 for CEU-938, respectively. The methods are thus compliant and considered as statistically valid.



835	0–2.1
934	0–1.1
938	0–0.4

835	2.1–3.8
934	1.1–2.2
938	0.4–3.7

835	3.8–12.7
934	2.2–7.3
938	3.7–12.4

835	12.7–1220
934	7.3–1484
938	12.4–1319

Fig. 2. Non-quantifiable range, semi-quantifiable range, and the analytical measurement range for the validation of CEU-835, CEU-934, and CEU-938.

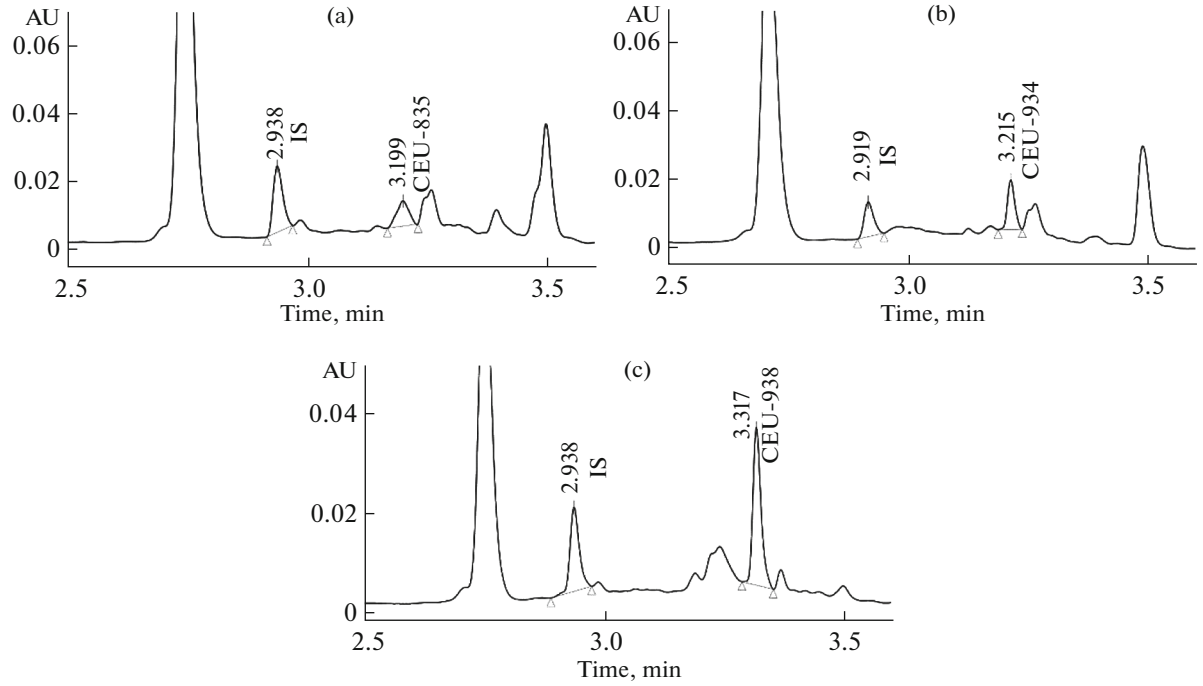


Fig. 3. Chromatograms of (a) CEU-835, (b) CEU-934, and (c) CEU-938 together with CEU-926 used as an IS at 2.8 μM of reconstituted samples from mice plasma.

The sensibility of the detection for CEU-835, CEU-934, and CEU-938 in the solvent and plasma matrices is shown in Table 6. These data will be used for calibration purposes and for the reproducibility of the method. The m_c must remain constant throughout the analysis of samples and it will be considered as a quality assurance parameter. The m_c for solvent and sample matrices are 11.831 and 2.13, 24.253 and 9.89, and 62.068 and 27.88 AU/ng mL^{-1} for CEU-835, CEU-934, and CEU-938, respectively.

The UHPLC-UV detection method is robust in the sample matrix at different wavelengths. Resolution and theoretical plate values for CEU-835, CEU-934, and CEU-938, as well as for the IS, are shown in Table 7. The R_s is valid if it has a higher value than 2.0 [13]. All R_s values at the selected wavelengths are higher than 2.0 and are thus considered valid. According to the system suitability testing limits, the theoretical plates must be at least 2000 for an acceptable chromatographic method [13]. All the selected wavelengths are thus suitable for analyzing CEU-835, CEU-934, and

Table 5. Conformity R values for IDL and MDL validation for CEU-835, CEU-934, and CEU-938

Standard candidate	IDL	MDL
CEU-835	8.20	4.43
CEU-934	4.04	7.23
CEU-938	4.97	4.18

CEU-938 in terms of N, as all of them are higher than 2000. Therefore, the novel method can be performed using different detection wavelengths. The lowest wavelength for the detection of CEU-835 is 250 nm, while the highest is 310 nm. For CEU-934 and CEU-938, the ranges of detection wavelengths are 260–310 and 273–310 nm, respectively.

Finally, the novel method is fast, reliable, and robust, and can be transferred for the quantification of PAIB-SOs in any complex biological matrix. This method included a more complex process of extraction but does not require radiolabeled molecules for the identification of the target compounds, which are advantages over previously developed HPLC/UHPLC methods for PAIB-SOs [3, 5].

CONCLUSIONS

A novel method for the detection of CEU-835, CEU-934, and CEU-938 was developed in plasma matrix of CD-1® IGS female mice using a UHPLC ACQUITY Arc system equipped with a 2998 UV/visible photodiode array detector. CEU-926 was selected and used as an IS for the evaluation of the validity of the method. The method is compliant in terms of IDL, MDL, and LOQ, and it was determined that the reliable detection in the method corresponds to the attended range of detection in animal models. Moreover, the R values that were calculated for all IDL and MDL meet the standards of the field and confirm that the methods are statistically valid for the analysis of PAIB-SOs. The sensibility varies from 2.13 to 62.068 AU/ng mL^{-1} in the different evaluated matrices, and the method is robust as it can be performed at a wide range of wavelengths that vary from 273 to 310 nm for all selected PAIB-SOs. In conclusion, this method is suitable for the detection and quantification of PAIB-SOs in CD-1® IGS female mice plasma. It can be used for their half-life evaluation in mice, and it can be an important methodological approach for their quantification in other biological matrices.

Table 6. Sensibility of the detection method in the pure solvent and sample matrices for CEU-835, CEU-934, and CEU-938

Standard candidate	m_c, AU/ng mL^{-1}	
	solvent	sample
CEU-835	11.831 ± 0.004	2.13 ± 0.01
CEU-934	24.253 ± 0.003	9.89 ± 0.02
CEU-938	62.068 ± 0.005	27.88 ± 0.02

Table 7. Resolutions and theoretical plates of CEU-835, CEU-934, CEU-938, and CEU-926 at selected wavelengths (λ)

Standard candidate	λ	R_s	$N_{PAIB\text{-}SOs}$	$N_{CEU\text{-}926}$
CEU-835	310	2.27	16034	8982
	300	2.21	14777	8685
	270	3.00	20943	19502
	260	3.20	20943	25472
	250	3.28	19980	30472
CEU-934	310	3.64	70580	10995
	300	3.95	51217	15910
	270	3.89	25857	26645
	260	4.15	36439	25161
CEU-938	310	4.99	27539	27011
	300	3.87	17260	15409
	275	3.50	17260	10315
	273	3.67	14707	14746

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal-related experiments were conducted in accordance with the Conseil canadien de protection des animaux (CCPA) and the Comité de protection des animaux de l'Université Laval (CPAUL) and its guidelines (https://www.services-recherche.ulaval.ca/conduite-responsable-et-ethique/protection-des-animaux). Animal protocols were approved by the CPAUL, Université Laval, Québec, Canada (Approval IDs 2018-014-1 and 2018-014-2).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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