[IJAP] Submission Acknowledgement

Yahoo/Inbox

Editor
From: ijap@innovareacademics.in
To: Caroline, Nathania Sie, Kuncoro Foe, Senny Yesery Esar

Tue, Jun 11, 2019 at 1:43 PM

Hello,

Maria Anabella Jessica has submitted the manuscript, "Characterization of pharmacokinetics of 2-(3-(chloromethyl)benzoyloxy)benzoic acid in rats " to International Journal of Applied Pharmaceutics.

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Editor

[IJAP] Your manuscript IJAP 34536: Revise and resubmit 4

Yahoo/Inbox

Editor IJAP
From: editor@ijaponline.org
To: Caroline, Nathania Sie, Kuncoro Foe, Senny Yesery Esar, Maria Anabella Jessica

Fri, Jul 5, 2019 at 12:05 PM

Dear Caroline, Nathania Sie, Kuncoro Foe, Senny Yesery Esar, Maria Anabella Jessica,

We have reached a decision regarding your submission to International Journal of Applied Pharmaceutics, "Characterization of pharmacokinetics of 2-(3-(chloromethyl)benzoyloxy)benzoic acid in rats " with reference no. IJAP 34536.

Article is quite interesting but it needs major revision

Comments-

For revision of your article see the following points

Minor Comments

- Format-: Revise article to make it strictly as per format of the Journal. Refer latest issue of the Journal for formatting..
- Abbreviations-: At the first appearance in the abstract and as well as in the text, abbreviations should be preceded by words for which they stand, for example, Cardio Vascular Disease (CVD) etc. These abbreviated forms should be used uniformly in the whole manuscript to maintain the consistency and uniformity.
- Abstract: Rewrite Abstract which should be structural (Divide it into- Objective, Methods, Results, and Conclusion).
- Symbol and units: It should be as per International System of Units (SI). See it in instructions to authors and follow accordingly and strictly.
- Errors: Grammatical and punctuation errors should be rectified. Authors are suggested to use smart tools like 1 checker, ginger, grammarly, white smoke, etc.
- Insert Table(s) and Figure(s) in Result and Discussion Section at appropriate place.
- Fig: Ensure that titles at x and y axis are in sentence case and bold.
- Ensure that biological names (plants/crude drugs/bacteria/fungus etc) are in italic in whole manuscript including reference also.
- References: References are out of format. Uniformity must be ensured in all the references. It should be made strictly as per Instructions to Authors. Journal's title should be non-italic, abbreviated without use of full stop.
- References: There is no need to mention issue number with volume. It should be provided only if it is supplement issue. Please correct accordingly.
- Pagination style is incorrect in references. Authors should refer any latest published article in IJAP. Digit appeared in starting page number should not be repeated in end page number. Ex. 12-5, 25-32, 125-7, 11456-62 etc.

[Few examples of references from journal:

Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6. **List the first six contributors followed by et al.**

Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994;102 Suppl 1:275-82.

Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.]

Major Comments

- Introduction: justify novelty and rationality of the study.
- Table footnotes: Authors must mention values of n, i.e. number of experiments, for example, 3, 4, 5 or 10 etc. Please mention that data given in mean±SD or mean±SEM in footnotes of each table.
- Authors should add/replace at least 2 references from International Journal of Pharmacy and Pharmaceutical Sciences and may be at least one from AJPCR, IJAP, IJCPR and JCR.
- See the attachment in the section "**Revisions**" for more comments and queries. Authors need to make the corrections according to these comments also while doing the revision

Editorial suggestive comment: Authors are suggested to cite references from the Journals of Innovare Academic Sciences (IAS) like International Journal of Pharmacy and Pharmaceutical Sciences (IJPPS) Asian Journal of Pharmaceutical and Clinical research (AJPCR), International Journal of Current Pharmaceutical Research (IJCPR), Journal of Critical reviews (JCR) etc. in this manuscript, only if it does not affect the write up of the manuscript in any way. Please avoid self-citation, provided necessary to cite, in any of the manuscript being communicated to any journal of IAS.

(All the changes made must be highlighted with RED coloured fonts or it should be done in track change mode).

-

Response to comments:

1. Authors are requested to make revision point to point and very strictly. Failure may cause its rejection.
2. Authors must give their response to the comments of reviewers at end of the revised copy of manuscript. If authors disagree with any comment they should record response with reason.

Note: Authors must send email to editor@ijaponline.org, after submission of revised article compulsorily with subject- "Revised article submitted for Round 1/2/3....." along with article reference no.

Please submit your revised article/manuscript as follows:

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With Best Regards
Editor
International Journal of Applied Pharmaceutics

Editor IJAP

[IJAP] Your manuscript IJAP 34536: Acceptance 3

Yahoo/Inbox

Editor IJAP
From: editor@ijaponline.org
To: Caroline, Nathania Sie, Kuncoro Foe, Senny Yesery Esar, Maria Anabella Jessica

Fri, Jul 26, 2019 at 1:55 PM

Dear Caroline, Nathania Sie, Kuncoro Foe, Senny Yesery Esar, Maria Anabella Jessica,

I am happy to inform you regarding your submission to International Journal of Applied Pharmaceutics, "Characterization of pharmacokinetics of 2-(3-(chloromethyl)benzoyloxy)benzoic acid in rats " that it has been recommended for publication after peer review.

I acknowledge you receipt of the registration fee by Swift for IJAP 34536.

Your article is now accepted for publication and your article is scheduled to be published in Vol 11, Issue 5, Sep 2019.

Citation of any published manuscript is always important for authors and journal also. Authors are requested to cite the publication of this manuscript in their future publication in other journals. Authors may extend the request to their colleagues also to cite. Further, explore the option of social media sharing on the Abstract page of the published article to showcase your publication.

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Editor
International Journal of Applied Pharmaceutics

Editor IJAP

RESPONSES TO REVIEWER'S COMMENTS

No	Comments	Responses
Minor Comments		
1	Format-: Revise article to make it strictly as per format of the Journal. Refer latest issue of the Journal for formatting	The writing of this article has been revised according to instructions to authors.
2	Abbreviations-: At the first appearance in the abstract and as well as in the text, abbreviations should be preceded by words for which they stand, for example, Cardio Vascular Disease (CVD) etc. These abbreviated forms should be used uniformly in the whole manuscript to maintain the consistency and uniformity.	The abbreviated form of 2-(3-(chloromethyl)benzoyloxy)benzoic acid has been revised namely 3CBB.
3	Abstract: Rewrite Abstract which should be structural (Divide it into- Objective, Methods, Results, and Conclusion).	The abstract has been written in structural form according to instructions to authors.
4	Symbol and units: It should be as per International System of Units (SI). See it in instructions to authors and follow accordingly and strictly.	The writing of symbol and units have been revised according to International System of Units (SI), for example mL to ml.
5	Errors: Grammatical and punctuation errors should be rectified. Authors are suggested to use smart tools like 1 checker, ginger, grammarly, white smoke, etc.	The grammatical and punctuation errors have been corrected using Grammarly.
6	Insert Table(s) and Figure(s) in Result and Discussion Section at appropriate place.	The tables and figures have been moved into the text at appropriate place.
7	Fig: Ensure that titles at x and y axis are in sentence case and bold.	The titles at x and y in the figures has been corrected.
8	Ensure that biological names (plants/crude drugs/bacteria/fungus etc) are in italic in whole manuscript including reference also.	It has been revised in italic according to the comment.
9	References: References are out of format. Uniformity must be ensured in all the references. It should be made strictly as per Instructions to Authors. Journal's title should be non-italic, abbreviated without use of full stop.	Writing of references has been corrected according to instructions to authors (see 'References' section).

10	References: There is no need to mention issue number with volume. It should be provided only if it is supplement issue. Please correct accordingly.	The issue number of journal has been deleted if it is not supplement issue (see 'References' section).
11	Pagination style is incorrect in references. Authors should refer any latest published article in IJAP. Digit appeared in starting page number should not be repeated in end page number. Ex. 12-5, 25-32, 125-7, 11456-62 etc.	The writing of page number in references has been corrected according to instructions to authors (see 'References' section).
Major Comments		
1	Introduction: justify novelty and rationality of the study.	The novelty and rationality of the study have been added in the 'Introduction' section. (see 'Introduction' section pharagraph 5 line 59-61).
2	Table footnotes: Authors must mention values of n, i.e. number of experiments, for example, 3, 4, 5 or 10 etc. Please mention that data given in mean±SD or mean±SEM in footnotes of each table.	The table footnotes have been added to Table 1 (line 197-203), Table 2 (line 210-211), Table 3 (line 235-239).
3	Authors should add/replace at least 2 references from International Journal of Pharmacy and Pharmaceutical Sciences and may be at least one from AJPCR, IJAP, IJCPR and JCR.	The reference from International Journal of Pharmacy and Pharmaceutical Sciences (line 315-318, 324-325, 327-329) and IJAP (line 311-313) have been added.
4	Authors need to make the corrections as written in the section "**Revisions**" for more comments and queries.	
a	Good research paper titles (typically 10–12 words long) use descriptive terms and phrases that accurately highlight the core content of the paper (e.g. Aim/objective of study, literary work evaluated, or the technology discussed).	The title of the article has been revised to '**COMPARATIVE PHARMACOKINETICS OF 2-(3-(CHLOROMETHYL)BENZOYLOXY)BENZOIC ACID AND ACETYLSALICYLIC ACID IN RATS'**.
b	Abstract summarizes the major aspects of the entire paper. Mention the abstract accordingly	The abstract has been revised (see 'Abstract' section).
c	A conclusion is not merely a summary of the topic covered; it should be more specific and clear as it offers new insight and creative approaches	The conclusion has been revised (see 'Conclusion' section).

COMPARATIVE PHARMACOKINETICS OF 2-(3-(CHLOROMETHYL)BENZOYLOXY)BENZOIC ACID AND ACETYLSALICYLIC ACID IN RATS

ABSTRACT

Objective: A new compound of salicylic acid derivative, namely 2-(3-(chloromethyl)benzoyloxy)benzoic acid (3CBB), was synthesized to find a compound exhibiting higher analgesic activity and smaller ulcer irritation than acetylsalicylic acid (ASA). Therefore, this study aimed to investigate the pharmacokinetics of this new compound in rats, following a single dose oral administration of 3CBB (45 mg/kg BW).

Methods: Plasma samples of 9 healthy rats were collected before and up to 3 h after its oral administration, following an 18 h fasting period. Plasma concentrations of 3CBB were determined using a validated HPLC-DAD assay. Pharmacokinetic parameters were determined using the compartment model technique. All experiments were carried out in triplicate.

Results: The pharmacokinetic parameters of 3CBB obtained were as follows: T_{max}= 28.9 ± 1.1 min, C_{max} = 0.57 ± 0.02 µg/ml, AUC_{total} = 66.3 ± 1.0 µg min/ml, K_{el} = 0.018 ± 0.002 min^{-1}, and $T_{1/2el}$ = 39.4 ± 3.9 min. The long elimination half-life and low C_{max} indicated that 3CBB was extensively distributed in the deep and very deep tissues. This confirmed the unique and special characteristics of a highly lipophilic compound like 3CBB (log P = 3.73).

Conclusion: 3CBB demonstrated a slower onset of action and longer elimination time from the body compared to ASA. Thus this new compound is a potential candidate to be developed as a new drug.

Keywords: 2-(3-(chloromethyl)benzoyloxy)benzoic acid; Pharmacokinetics; Rat; HPLC-DAD.

INTRODUCTION

Acetylsalicylic acid (ASA, aspirin) has been used as a non-steroidal anti-inflammatory drug and antiplatelet agent for decades. After oral administration, ASA will be absorbed rapidly, mostly in the upper small intestine at the low pH. The absorption of ASA follows first-order kinetics with the absorption half-life lies within the range from 5 to 16 min [1]. ASA is highly bound by plasma protein (99%). It is hydrolyzed primarily in the liver, yielding salicylic acid. This is subsequently conjugated with glycine or glucuronic acid, and the conjugate is excreted mostly in urine [2]. The pharmacokinetic profile of ASA in rats after oral administration (40 mg/kg BW) was reported, with the following parameters area under the plasma concentration versus time curve from zero to infinity ($AUC_{0-\infty}$) = 152.2 µg min/ml, elimination half-life ($T_{1/2}$) = 5.66 min, peak plasma concentration (C_{max}) = 9.74 µg/ml, and time to reach maximum drug concentration in plasma (T_{max}) = 7 min [3]. Meanwhile, other study reported the pharmacokinetics of ASA in human plasma after oral administration (500 mg/70 kg BW), with the following parameters: $AUC_{0-\infty}$ = 5.12 mg h/ml, $T_{1/2}$ = 0.422 h, C_{max} = 4.84 µg/ml, T_{max} = 0.5 h [4].

Despite its ability to inhibit inflammation and its cardioprotective effect, many studies reported the harmful effect of ASA such as gastric irritation [5, 6].

To maintain the benefit of ASA and minimize its harmful effect, we have modified the structure of salicylic acid, by replacing the acetyl group with the benzoyl group, yielding a compound known as 2-(3-(chloromethyl)benzoyloxy)benzoic acid (3CBB) (Fig. 1).

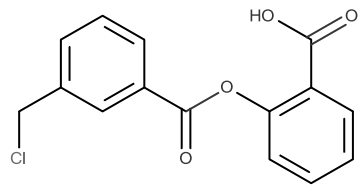


Fig. 1: Chemical structure of 3CBB.

The benzoyl group is more lipophilic than the acetyl group, and hence increasing the ability of the molecule to penetrate the membrane which in turn will increase its pharmacological activity. In our previous study, we investigated the analgesic, toxicity and antiplatelet effects of 3CBB. This study demonstrated that our new compound is more active and less toxic than ASA [7].

To our knowledge, until now there is no study has reported the pharmacokinetic parameters of 3CBB. The pharmacokinetic profile may give a description of the onset of action, duration of action, and any side effects related to the 3CBB level in the systemic circulation.

In this paper, we investigated the blood levels of this new compound with validated High Performance Liquid Chromatography UV method – Diode Array Detector (HPLC-DAD) [8,9]. This study aimed to describe the pharmacokinetic profile of 3CBB in rats following oral administration, as a part of its development as a new drug.

MATERIALS AND METHODS

Chemicals and reagents

The material used in this study was 3CBB, which was synthesized in our laboratory. The purity and identification of this compound were determined by using TLC, IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR and EDX [7].

Methanol of HPLC grade was obtained from Merck (Germany), whereas acetonitrile, phosphoric acid and potassium dihydrogen phosphate of pro analytical grade were purchased from E. Merck (Germany). Distilled and deionized water was obtained from Otsuka (Indonesia), and *pulvis gummi arabicum* was purchased from Brataco (Indonesia).

Instrumentation

The HPLC system employed in this study was Hitachi L-2130 (Japan), 100-µl injector (Rheodyne 7725, USA), DAD (Hitachi L-2455, Japan), and LiChroCART 250-4, LiChrospher 100 RP-18 (5 µm, 4×250 mm, Germany) as a stationary phase. Sample analysis was conducted isocratically using a mixture of methanol : phosphate buffer pH 4 (4:6, v/v) as a mobile phase at the flow rate of 1.0 ml/min.

The other instruments used were pH meter (Metrohm 620, Switzerland), centrifuge (Zentrifugen Hettich EBA 8S, Germany), vortex (Thermolyne, Iowa), sonicator and degassing apparatus (Branson 1210, USA), filter holder and Whatmann filter paper with 0.45 µm pore size (Millipore), analytical balance (Denver, India), and micropipette (Socorex, Swiss).

Animals

Nine healthy Wistar rats (*Rattus norvegicus*), weighing 200 g, aged 2-3 mo were obtained from Pusvetma (Indonesia). Experimental animals were housed in a temperature-controlled room (20-24°C) with a 12 h light/dark cycle. They were allowed to consume food and water *ad libitum*. All experiments performed in this study obtained the ethical clearance, as approved by The University of Gadjah Mada Committee on the Use and Care of Animals (No. 00090/04 / LPPT / VII / 2017), acting on behalf of the Indonesian Government.

Preparation of stock and working standard solutions

A stock standard solution of 3CBB at a concentration of 4 mg/l was prepared by weighing and dissolving it in methanol. A solution of salicylic acid at a concentration of 0.24 mg/l in methanol was used as an internal standard. Working standard solutions were prepared by diluting an appropriate volume of the stock standard solution, yielding 0.15 – 4 mg/l.

For the accuracy and precision study, an aliquot of the stock standard solution was diluted with 100 µl of blank plasma to yield a series of concentrations of 3CBB in the range of 0.24-3.0 mg/l. Fifty microlites of the internal standard solution was added into the aliquots and the resulting working standard solutions were transferred into 1.5 ml tubes. Afterward, 300 µl of acetonitrile was added into each tube, and then they were vortexed for 10 min and centrifuged at 3500 rpm for 2 min. The filtrate was collected and evaporated to dryness under nitrogen gas. The residue was dissolved in 50 µl of mobile phase and was used as a sample.

Validation study

HPLC assay condition for bioanalytical method validation according to US FDA guidelines have been established [10]. The selectivity of this HPLC assay was tested by injecting samples, consisting of six blank plasma obtained from six rats. The presence of the 3CBB at a particular retention time was evaluated [11,12].

A calibration curve was determined on each day of the 6 day validation period. Each HPLC run consisted of blank plasma, a zero calibrator (blank plasma with internal standard), and non-zero calibrators, comprising six working standard solutions at various concentrations ranging from 0.15 – 4 mg/l.

The accuracy and precision (intra-day and inter-day variability) study were performed in triplicate during 6 d validation period, employing five concentrations as quality control (QC) standard levels, ranging from 0.24-2.64 mg/l. The accuracy and precision of this HPLC assay were expressed as Relative Error (%) and Relative Standard Deviation (%), respectively.

The stability of analyte in plasma during storage was performed, by analyzing the concentrations of this compound at three conditions, namely fresh-thaw after 0 h, 4 h, and freeze-thaw (24 h at -20 ^{0}C). This study was performed in triplicate employing four QC concentrations (0.25, 0.5, 3, 4 mg/l) that were freshly prepared.

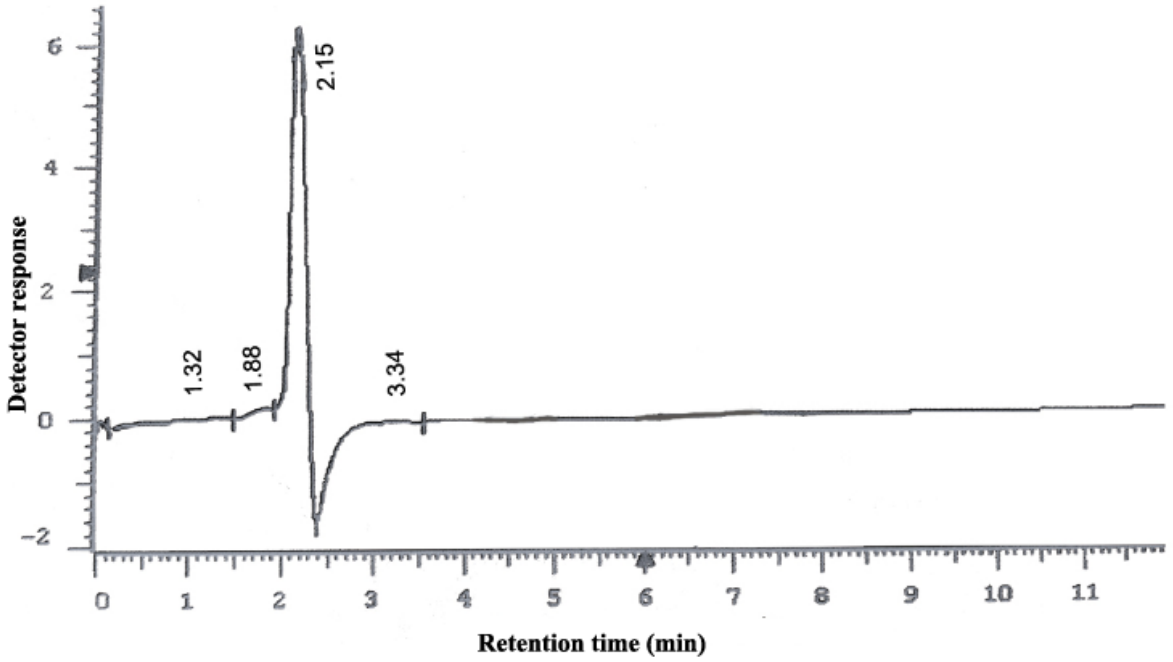
Pharmacokinetic study

Prior to treatment, rats have fasted for 18 h. The rats were anesthetized with ketamine intraperitoneal (100 mg/kg BW) [13]. After 10 min, animals were treated orally with 3CBB suspended in 1 ml 3% *pulvis gummi arabicum*, with a dose of 45 mg/kg BW. Blood samples were withdrawn from vena caudalis and collected into heparinized tubes, prior to dosing (time 0) and at pre-determined time intervals as follows 5, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90, 120, and 180 min. The collected blood sample was immediately centrifuged at 10,000 rpm for 10 min to obtain plasma and then was stored at -20 ^{0}C prior to analysis. Plasma concentrations were determined using a validated HPLC assay. Pharmacokinetic parameters were determined using a compartment model technique. All experiments were carried out in triplicate.

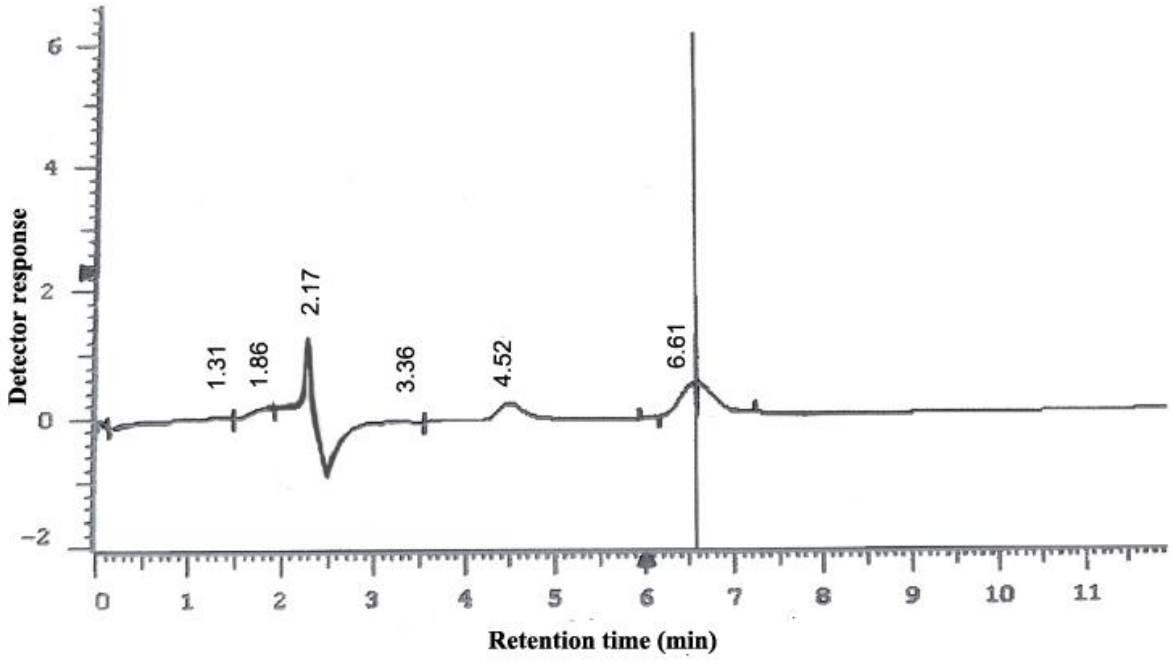
RESULTS AND DISCUSSION

Optimization of HPLC condition

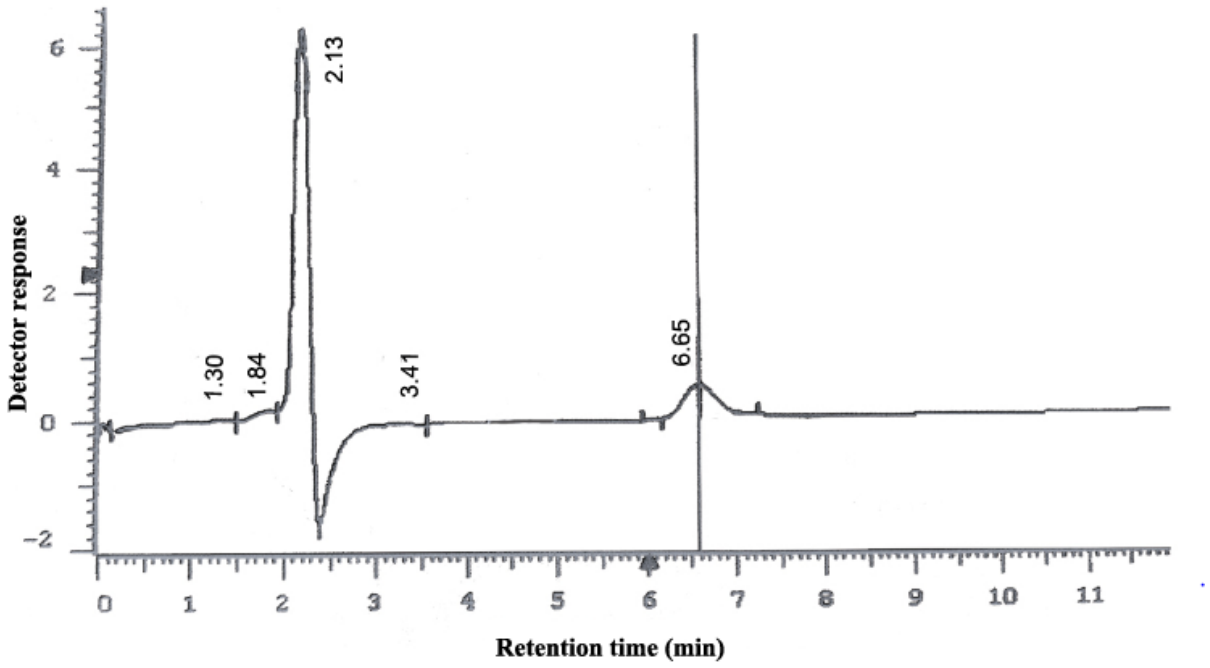
HPLC analytical method was performed isocratically using a mixture of methanol: phosphate buffer pH 4 (4:6, v/v) as a mobile phase at the flow rate of 1.0 ml/min. This was selected as the best HPLC assay condition, as it gave the best peak shape and resolution of peaks associated with salicylic acid and 3CBB in plasma. A typical HPLC chromatogram demonstrating the peaks associated with blank plasma, 3CBB, and internal standard is shown in Fig. 2. No interference among peaks was observed, indicating the selectivity of this HPLC assay.


Detector response
Retention time (min)
1.32
1.88
2.15
3.34

(a)


Detector response
Retention time (min)
1.31
1.86
2.17
3.36
4.52
6.61

(b)



(c)

Fig. 2: Typical chromatogram profiles of: (a) Blank plasma, (b) Internal standard and 3CBB in plasma, (c) 3CBB in plasma. Notes: mobile phase was methanol : phosphate buffer pH 4 (4:6, v/v) with the flow rate of 1 mL/min; stationary phase/column was LiChrospher 100 RP-18 (5 µm, 4 × 250 mm, Germany); detector was Diode Array Detector (DAD, Hitachi L-2455, Japan) at a wavelength of 231 nm; retention times 4.52 min for internal standard and 6.6 min for 3CBB.

A calibration curve was obtained using six concentrations covering the linear range, in which each concentration was analyzed in six replicates. It was found that peaks associated with zero calibrators (blank plasma with endogenous substance) and internal standard did not interfere with the peak associated with 3CBB. The selected linear regression equation for 3CBB used in this study was y = 251376 x -22538, demonstrating a good correlation between concentration (x) and peak area (y) $r_{calculated} = 0.99638 > r_{table} = 0.8114$). In addition, $F_{calculated} = 0.145 < F_{table} = 4.14$, indicating an insignificant difference between the calibration curves generated on each day of the 6 day validation period.

The LOQ (Limit of Quantitation) and LOD (Limit of Detection) values were found to be 0.1410 mg/l and 0.0423 mg/l, respectively. Therefore this method was relatively sensitive to determine 3CBB.

The intra-day and inter-day variability of accuracy and precision studies of 3CBB are shown in Table 1. The relative error of intra-day and inter-day variability were found to be 0.32 – 1.33 % and 0.46 – 1.71 %, respectively. Meanwhile, the respective relative standard deviation of intra-day and inter-day variability were 0.05 – 0.62 % and 0.12 – 0.80 %. Thus this HPLC assay was relatively accurate and precise to be employed in this study. The mean recovery of 3CBB in rat plasma was found to be 100.94% ± 0.35%.

Table 1: The intra-day and inter-day variability of accuracy and precision studies of 3CBB.

Nominal Concentration (µg/ml)	Intra-day Variability[c]		Inter-day Variability[d]		Recovery[e] (%) (Mean ± RSD[b])
	Accuracy (RE[a], %)	Precision (RSD[b], %)	Accuracy (RE[a], %)	Precision (RSD[b], %)	
0.24	1.18	0.62	1.71	0.80	101.53 ± 0.75
0.72	0.86	0.27	1.27	0.26	101.14 ± 0.32
1.20	1.33	0.30	1.53	0.68	101.46 ± 0.57
1.68	0.44	0.17	0.58	0.14	100.53 ± 0.16
2.16	0.38	0.08	0.65	0.15	100.56 ± 0.18
2.64	0.32	0.05	0.46	0.12	100.41 ± 0.12

[a] RE: Relative Error (%) = (actual concentration – nominal concentration)/nominal concentration × 100%

[b] RSD: Relative Standard Deviation (%) = Standard Deviation / mean of actual concentration × 100%

[c] n = 3

[d] n = 18

[e] n = 21

The stability study of 3CBB in rat plasma is shown in Table 2. The sample was found to be stable during storage at -20 ^{0}C for 24 h and thus it could be used for pharmacokinetic study.

Table 2: Stability study of 3CBB in rat plasma.

Nominal Concentration (µg/ml)	Fresh-Thaw Stability[a]		Freeze-Thaw Stability[a] (-20 ^{0}C, 24 h) (Mean ± RSD[b])
	0 h (Mean ± RSD[b])	4 h (Mean ± RSD[b])	
0.25	101.97 ± 1.56	101.08 ± 0.68	100.25 ± 0.22
0.5	103.01 ± 1.25	102.04 ± 1.62	100.82 ± 0.88
3	106.42 ± 1.17	104.11 ± 1.28	102.10 ± 1.20
4	106.90 ± 1.19	105.81 ± 1.20	103.41 ± 1.15

[a] n = 3

[b] RSD: Relative Standard Deviation (%)

Pharmacokinetic study

The pharmacokinetic profile of 3CBB in rat plasma is shown in Fig. 3. It exhibited a non-linear elimination phase, the plasma concentration of 3CBB increased slowly in the first 40 min and then rose and declined sharply within 40 – 60 min after oral administration. A tail-off profile was observed afterward. Previous studies [3, 14] reported that the pharmacokinetic profile of ASA increased dramatically in the first 5 min and then declined rapidly, whereas the concentration of its degradation product, salicylic acid constantly increased.

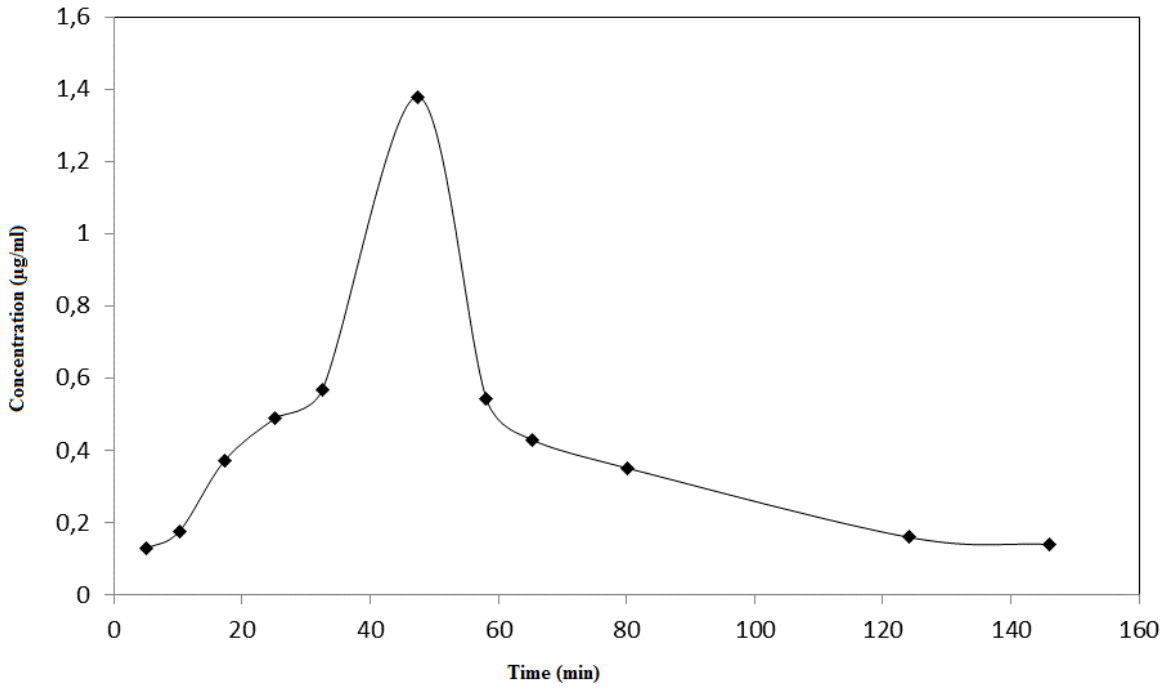


Fig. 3: A typical pharmacokinetic profile of 3CBB in rat plasma following oral administration (45 mg/kg BW).

The value of T_{max} is inversely proportional to the value of the absorption rate constant (k_a). If there is an increase in k_a, it would cause a decrease in the value of T_{max} and *vice versa*. The obtained T_{max} value of 3CBB was 28.9 ± 1.1 min (Table 3), which is much longer than ASA (7 min) as reported previously [3]. This study revealed that our new product exhibited a slower onset of action than ASA. This might be associated with its higher lipophilic properties and the stability of this product which was not easily hydrolyzed to yield salicylic acid.

Table 3: Calculated pharmacokinetic parameters of 3CBB in rat plasma after oral administration (45 mg/kg BW).

Parameter	3CBB[a,d] (Mean ± RSD)	Acetylsalicylic acid	
		[4][b]	[3][c]
K_{el} (min^{-1})	0.018 ± 0.002	-	-
$T_{1/2\ el}$ (min)	39.4 ± 3.9	25.32 ± 7.93	5.66 ± 1.27

T_{max} (min)	28.9 ± 1.1	30	7.0 ± 1.6
C_{max} (µg/ml)	0.57 ± 0.02	4.84 ± 1.73	9.74 ± 1.09
$AUC_{0-\infty}$ (µg min/ml)	66.3 ± 1.0	307.2 ± 110.90	152.2 ± 23.3

[a] 2-(3-(chloromethyl)benzoyloxy)benzoic acid.

[b] Pharmacokinetic study was performed in humans at a dose of 500 mg [4].

[c] Pharmacokinetic study was performed in Sprague Dawley rat at the dose of 40 mg/kg BW [3].

[d] n = 9

The following pharmacokinetic parameters were determined, including the time to reach the maximum concentration (T_{max}), maximum concentration (C_{max}), and the area under the curve of a plasma concentration *versus* time from 0 to infinity ($AUC_{0-\infty}$).

The maximum concentration of one particular drug in the systemic circulation highlights the intensity of the drug. C_{max} is correlated with the volume of distribution (Vd). If there is an increase in Vd value, the value of C_{max} will decrease and vice versa. Vd is very useful for estimating the relative amount of drug within the central or peripheral compartment. A large value of Vd indicates the higher amount of drug accumulating in the peripheral tissue compared to that in the central compartment. The C_{max} value of this new compound was found to be 0.57 ± 0.02 µg/ml (n = 3, Table 3). The low C_{max} is associated with a large volume of distribution. Thus it could be hypothesized that this new compound was extensively distributed in the deep and very deep tissues. This phenomenon is commonly expressed by a highly lipophilic compound. This new compound, 3CBB, was reported to be more lipophilic (log P = 3.73) than ASA (log P = 1.21) as reported in our previous study [7].

The AUC_{total} of 3CBB was found to be 66.3 ± 1.0 µg min/ml (Table 3).

Elimination kinetics of one particular drug can be described by the parameter of elimination half-life ($T_{1/2el}$), the time required to reduce the blood drug level to half. Drugs can be cleared from the body through two main pathways namely kidneys and liver. Metabolism is a conversion process of the chemical structure of a drug in the body. In general, metabolism will convert the drug molecules to a more polar compound, that is more soluble in aqueous than fatty tissues, and

hence it is more easily excreted through the kidneys. Metabolism reaction of the drug is facilitated by the presence of an enzyme, to yield an inactive compound. However, there are some metabolites that are more active and more toxic than their parent drugs. In this study, the $T_{1/2el}$ value obtained was 39.4 ± 3.9 min, which is much longer than the respective values of ASA 5,66 min in rats [3] and 25.32 min in humans [4]. Therefore 3CBB may require a much longer time for its elimination, compared to ASA.

CONCLUSION

This new compound, 3CBB, exhibited the slower onset of action and longer elimination time from the body, compared to ASA. This is in agreement with the lipophilic properties of 3CBB compared to ASA. Thus this new compound, considering its pharmacological and toxicological properties, is a potential candidate to be developed as an alternative drug to substitute ASA.

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