

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/318453571>

# Validation and modification of HPLC method for monitoring acetaminophen plasma concentrations in patients with chronic pancreatitis

Article · July 2017

CITATIONS

0

READS

33

5 authors, including:



**Mohamed Abouzid**

Poznan University of Medical Sciences

14 PUBLICATIONS 13 CITATIONS

[SEE PROFILE](#)



**Hanna Urjasz**

Poznan University of Medical Sciences

28 PUBLICATIONS 146 CITATIONS

[SEE PROFILE](#)



**Krystian Adrych**

Medical University of Gdansk

105 PUBLICATIONS 519 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Vitamin D metabolites in Cardiovascular patients [View project](#)

## **Validation and modification of HPLC method for monitoring acetaminophen plasma concentrations in patients with chronic pancreatitis**

**Mohamed Abouzid<sup>1</sup>, Hanna Urjasz<sup>1</sup>, Magdalena Siepsiak-Połom<sup>2</sup>, Krystian Adrych<sup>2</sup>, Edyta Szalek<sup>1</sup>**

<sup>1</sup>Department of Clinical Pharmacy and Biopharmacy, Medical University of Poznań, Poznań, Poland

<sup>2</sup>Department of Gastroenterology and Hepatology, Medical University of Gdańsk, Gdańsk, Poland

### **Summary**

**Background.** Pancreatitis is the inflammation of the pancreas. The inflammation may occur suddenly and resolve in a few days with the treatment in case of acute pancreatitis (AP). Chronic pancreatitis (CP) is a persistent inflammation of the pancreas that results in irreversible morphological changes and impairment of both exocrine and endocrine functions. Abdominal pain is a typical symptom in both acute and chronic pancreatitis. Therefore, the application of analgesics to this group of patients is a part of complex pharmacotherapy. **Material and methods.** A simple, precise, specific, and accurate HPLC (High Performance Liquid Chromatography) method was developed for the determination of acetaminophen (AAP) in human plasma. The chromatography was set on XTerra® RP C18, 150×4.6 mm, 3.5 µm column. The UV detector was set at 254 nm for AAP and phenacetin (internal standard). The injection volume was 2 µL. The mobile phase comprised phosphate buffer pH 3.5 and methanol in the ratio of 80:20 with flow rate of 1 mL/min. **Results.** The calibration for AAP was linear in the range 1.0-30.0 µg/mL. Lower limit of quantification (LLOQ) was 1.0 µg/mL. The results for quality control samples (QCs) at concentrations of 1, 6, 15, and 25 µg/mL were less than 15% indicating good intra- and inter-day precision. Blood samples from patients with chronic pancreatitis were collected within 8 h after the drug intravenous administration (1000 mg). Pharmacokinetic (PK) parameters for AAP were estimated by non-compartmental method. **Conclusion.** The proposed HPLC-UV method is suitable for routine analysis in patients. (*Farm Współ 2017; 10: 3-10*)

*Keywords: acetaminophen, HPLC, validation, chronic pancreatitis*

### **Introduction**

Pancreatitis is the inflammation of the pancreas, which could be either acute or chronic [1]. Chronic pancreatitis (CP) is a progressive and permanent destruction of the pancreas leading to by exocrine and endocrine insufficiency and, often, chronic disabling pain [2]. The etiology of chronic pancreatitis is multifactorial [3,4]. Chronic alcohol use considers for 70 percent of the cases of chronic pancreatitis in adults, and most patients have consumed more than 150 g of alcohol per day over six to 12 years [5,6]. Moreover, the risk factors of CP include nicotine consumption, hereditary factors, efferent duct obstructions, immunological factors or rare metabolic disorders [4]. Patients with CP typically present with epigastric or left upper

quadrant pain which could be relieved by analgesics, e.g. acetaminophen [7].

Acetaminophen (paracetamol) is an analgesic and antipyretic drug. It is a weak prostaglandin inhibitor and possesses no significant anti-inflammatory effect [8,9]. *In vitro*, AAP is a weak inhibitor of both cyclooxygenases: COX-1 and COX-2, and inhibits additional form of COX, COX-3 [10]. The pharmacokinetics (PK) of AAP are linear. It is readily absorbed from the gastrointestinal tract with the peak plasma concentrations occurring about 10 to 60 minutes after oral administration [11]. The distribution of AAP occurs into most body tissues. At usual therapeutic doses, plasma protein binding is negligible but it with increases increasing doses. The volume of distribution

( $V_d$ ) of AAP is approximately 1 L/kg and 10-20% of circulating AAP is bound to plasma protein [12,13]. AAP is metabolised in the liver predominantly resulting in two major metabolites, sulphate and glucuronide conjugates. A small fraction (< 4%) is metabolised by cytochrome P450 to a reactive intermediate N-acetyl benzoquinone imine (NAPQI) [14]. Under normal conditions of use, NAPQI is rapidly detoxified by reduced glutathione. It conjugates with cysteine and mercapturic acid and is eliminated with the urine [14]. At therapeutic doses, the major isoform of P450 in human liver (CYP3A4), contributes to the production of the cytotoxic metabolite. For very high, supratherapeutic plasma concentrations (1500 mg/L) of AAP, the 2E1 and 1A2 isoforms may also be involved [11]. The metabolites of AAP are mainly excreted in the urine. 90% of the dose administered is excreted in 24 hours mainly as glucuronide and sulphate conjugates (60-80% and 20-30% respectively). Less than 5% of AAP is eliminated unchanged [12]. It is necessary to study the PK parameters in AP and CP because AP is one of the most common diseases of the gastrointestinal tract and it leads to enormous emotional, physical, and financial burdens [15,16]. In addition, the knowledge about drug absorption in CP is still limited [17].

The changes of drug PK are depending on the absorption which may be decreased in case of pancreatitis especially in CP, because it is well recognized that pancreatitis patients suffer from malabsorption [18,19]. The aim of the study was validation and modification of analytical method for monitoring acetaminophen plasma concentrations in patients with CP.

## Material and methods

### Chemicals and reagents

Working standards of acetaminophen and phenacetin – the internal standard (IS), were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. All chemicals and reagents used were of HPLC grade  $\geq 98\%$  and were purchased from Merck, Germany. Milli-Q grade (Millipore, Bedford, USA) water was used in all cases. Blank human plasma was obtained from the Blood Bank (Poznań, Poland) and stored at  $-22^\circ\text{C}$  prior to use. Patients' blood samples were collected at the Department of Gastroenterology and Hepatology, University of Medical Sciences, Gdańsk. The study was approved by the Ethics Committee in Gdańsk (No: NKBBN/221/2015).

### Chromatographic condition

The HPLC method reported by Helmy et al [20] was modified to fulfill our objectives. The validation of the method was done at the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznań.

The analysis was performed using a HPLC system equipped with a dual-wavelength absorbance detector and an autosampler (Waters 2695 Alliance, Milford, USA). Data analysis program Empower Pro, No 1154 Waters, Milford, USA was used for data acquisition and processing. Chromatographic separation was accomplished with a XTerra® RP C18 column (150 mm  $\times$  4.6 mm, 3.5  $\mu\text{m}$  particle size; Waters®, Ireland). The mobile phase comprised 0.1 M sodium dihydrogen phosphate buffer (pH adjusted to 3.5 with phosphoric acid) and methanol (80:20 v/v); the elution was isocratic at ambient temperature with a flow rate of 1 mL/min. The injection volume was 2  $\mu\text{L}$ . The UV detector was set at 254 nm for AAP and IS (phenacetin). The peak areas were calculated using the data analysis program Empower Pro software. The examples of chromatograms are presented in Figures 1-2.

### Standard solutions

The preparation of the stock solutions of 1000  $\mu\text{g/mL}$  of AAP was made by a proper dissolution of AAP substance into methanol. The stock solutions were used to prepare 12 standard solutions of AAP by diluting in methanol. Standard solutions concentrations were 10, 20, 40, 50, 80, 100, 120, 160, 180, 200, 250 and 300  $\mu\text{g/mL}$ . 100  $\mu\text{L}$  of working solution of AAP and 50  $\mu\text{L}$  IS were added to a final volume of 0.35 mL plasma to form a set of calibration standards with concentrations of 1, 2, 4, 5, 8, 10, 12, 16, 18, 20, 25 and 30  $\mu\text{g/mL}$  used to prepare matrix based samples. Quality control samples (QC) were prepared at 1, 2, 6, and 15  $\mu\text{g/mL}$  for AAP. A working IS solution containing 1.0 mg/mL phenacetin dissolved in methanol was prepared. All plasma solutions were stored at  $-22^\circ\text{C}$  until assay.

### Sample preparation

All blood samples were collected in tubes containing heparin. After centrifugation, plasma samples were conveyed to plastic tubes and stored frozen at  $-22^\circ\text{C}$  until examination. To perform the extraction of drugs from plasma, 450  $\mu\text{L}$  of plasma was added to a test tube containing 50  $\mu\text{L}$  IS accompanied by 500  $\mu\text{L}$  of

methanol in a polytetrafluoroethylene microfuge tube. The tube was vortexed for 1 min and then centrifuged at 7000 rpm for 6 min. The supernatant was separated, and 2  $\mu\text{L}$  was injected into the HPLC. All procedure steps were carried out at room temperature.

### Pharmacokinetics

PK parameters were calculated using software Phoenix™ WinNonlin® v. 6.3, Certara L.P.) based on non-compartmental kinetics. For each patient the following PK parameters for AAP were calculated: area under the plasma concentration-time curve from time zero to infinity ( $\text{AUC}_{\text{inf}}$ ), area under the plasma concentration-time curve from zero to the time of last measurable concentration ( $\text{AUC}_{\text{last}}$ ), maximum observed plasma concentration ( $C_{\text{max}}$ ), half-life in elimination phase ( $t_{0.5\text{kel}}$ ), clearance (Cl), mean residence time (MRT), area under the first moment curve from zero to the time of last measurable concentration ( $\text{AUMC}_{\text{last}}$ ), volume of distribution ( $V_d$ ).

## Results

### Assay Validation

#### Linearity

The following regression equation was found by plotting the peak area ratios of AAP to the IS (y) versus the acetaminophen concentrations (x) expressed in  $\mu\text{g/mL}$ :  $y = (1.64 \pm 0.43) \cdot x - (2.51 \pm 0.54)$ . The value of the coefficient  $r^2$  ( $>0.9976$ ) obtained for the regression line demonstrates the good relationship between peak area ratios of AAP to the IS and concentration of acetaminophen. The calibration for AAP was linear in the range 1.0-30.0  $\mu\text{g/mL}$ .

#### Limits of quantification

The lower limit of quantification (LLOQ) was 1  $\mu\text{g/mL}$  for AAP. In addition, LLOQ accuracy was within 20% of the nominal spiked concentration.

#### Selectivity and matrix effects

A 450  $\mu\text{L}$  of standardized plasma was used. Next, the blank samples were prepared to HPLC analysis according to the description presented above for calibration samples. There weren't detectable interfering peaks observed at the retention times of AAP that exceed the limit approved by EMA.

### Precision and accuracy

The results proved good precision and accuracy over the concentration range 1–30  $\mu\text{g/mL}$ . The within-day precision and accuracy were assessed by analyzing each quality control (QC) samples 4 times on the same day, while between-day precision and accuracy were assessed by calculating the overall QCs (at least 3 replicates at each concentration per analytical run) that were assayed during the study period. The coefficient of variation (CV %) of the measured concentrations was used to determine the precision of the assay.

$$\text{CV [\%]} = \text{Standard Deviation/Average} \times 100\%$$

The accuracy of the assay was defined as the absolute mean value of the ratio of the back calculated mean values of the unknown samples and their nominal values, expressed as a percentage.

$$\text{Accuracy [\%]} = \text{Absolute mean value/Nominal value} \times 100\%$$

The analysis run was accepted if at least 2 out of 3 QCs samples were within 20 % of the nominal concentration. The within- and between-day precision and accuracy of the 3 QCs and LLOQ of AAP in human plasma were listed in tables (I-VI). The %CV for the QCs and the LLOQ was within 10%, and the accuracy was ranged between 103.45 and 110.0%.

### Stability

Freeze-thaw, short-term and long-term stabilities were tested for the 3 QC's with 3 determinations for each (tables V and VI). Stability of AAP after 3 freeze-thaw cycles of plasma samples showed that it was stable when subjected to these conditions. Short-term stability prevailed unchanged up to 24 h at ambient temperature (25.7°C). The long-term stability was found to be constant over a period of 1 month at the storage condition of  $-22^\circ\text{C}$  in the refrigerator.

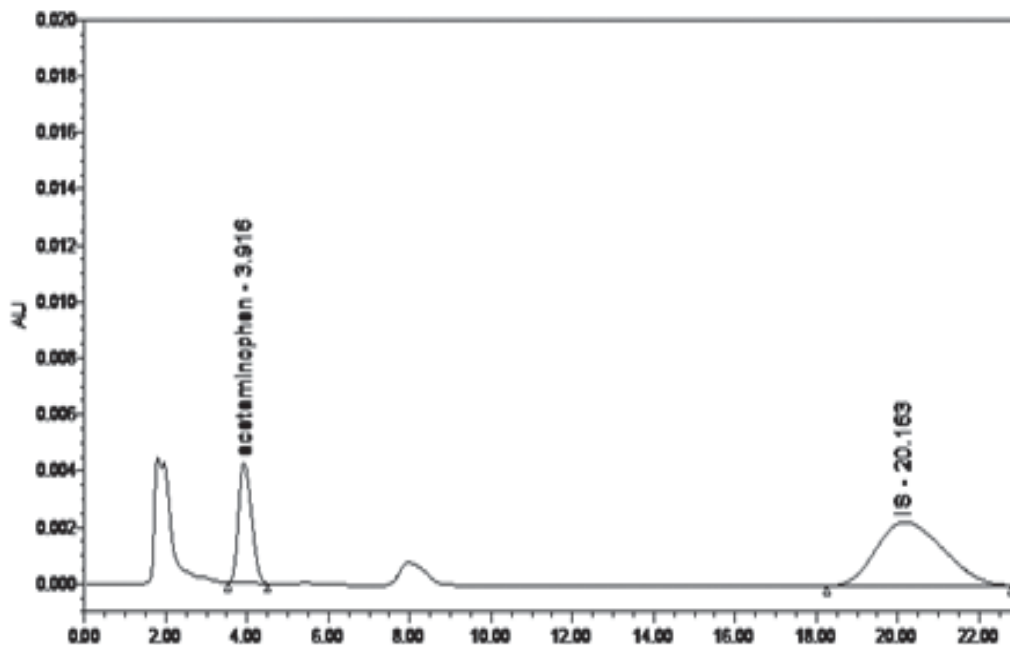


Figure 1. Chromatogram of acetaminophen in plasma at the concentration 15 µg/mL (IS – internal standard)

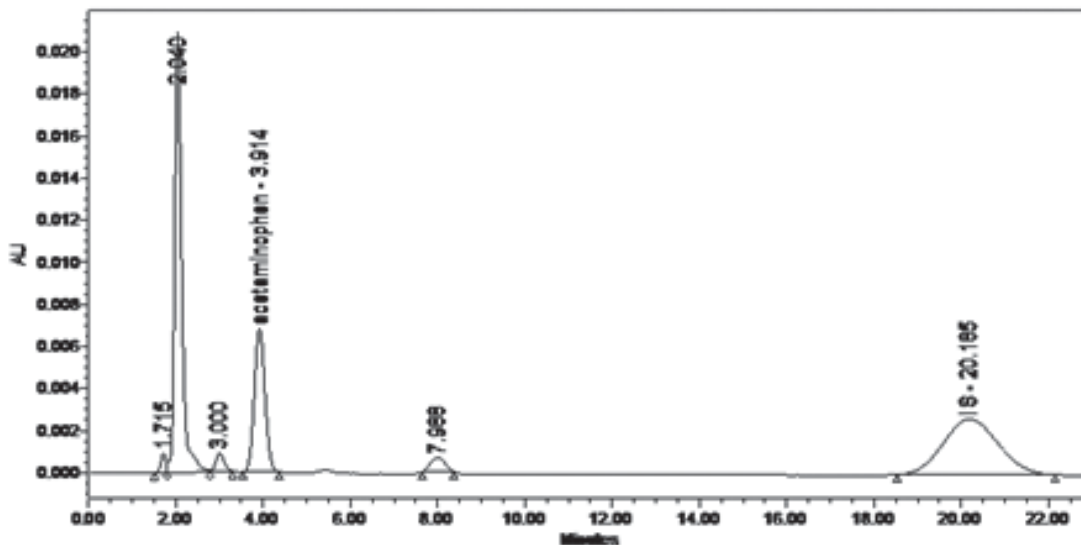


Figure 2. Example of chromatograph from patient's plasma (acetaminophen concentration: 22.17 µg/mL)

Table I. Precision within run

C [µg/mL]	C <sub>avg</sub> [µg/mL]	SD	CV [%]
1	1.10	0.04	3.26
6	5.93	0.14	2.50
15	15.52	0.46	3.01
25	25.62	0.44	1.73

Table II. Precision between run

C [µg/mL]	C <sub>avg</sub> [µg/mL]	SD	CV [%]
1	1.18	0.06	5.76
6	5.78	0.31	5.44
15	15.39	0.34	2.23
25	26.59	0.70	2.63

Table III. Accuracy within run

C [µg/mL]	C <sub>avg</sub> [µg/mL]	Accuracy [%]
1	1.10	110.00
6	6.53	108.83
15	15.52	103.45
25	25.62	102.51

Table IV. Accuracy between run

C [µg/mL]	C <sub>avg</sub> [µg/mL]	Accuracy [%]
1	1.17	117.00
6	5.78	96.33
15	15.39	102.60
25	26.59	106.384

Table V. The freeze and thaw stability

C [µg/mL]	C <sub>avg</sub> [µg/mL]	C <sub>freezing</sub> [µg/mL]	Stability [%]
2	2.14	2.25	95.11
6	6.53	6.18	105.66
15	15.52	15.10	102.78

Table VI. Long term stability of the analytes in matrix stored in the freezer (1 month)

C [µg/mL]	C <sub>avg</sub> [µg/mL]	C <sub>1 month</sub> [µg/mL]	Stability [%]
2	2.14	2.00	106.50
6	6.53	6.99	93.40
15	15.52	17.24	90.00

Table VII. Pharmacokinetic parameters of acetaminophen (1000 mg, *i.v.*) in patients with pancreatitis (n = 9)

Subject	K <sub>el</sub> [h <sup>-1</sup> ]	t <sub>0.5kel</sub> [h]	C <sub>max</sub> [µg/mL]	AUC <sub>last</sub> [(µg·h)/mL]	AUC <sub>inf</sub> [(µg·h)/mL]	V <sub>d</sub> [L]	CL [L/h]	AUMC <sub>last</sub> [(µg·h <sup>2</sup> )/mL]	MRT [h]
1	0.214	3.24	15.14	33.44	41.07	80.65	29.91	90.17	4.55
2	0.237	2.93	20.70	21.63	24.21	87.72	46.22	41.05	2.99
3	0.217	3.19	6.39	19.19	23.92	138.38	52.12	50.95	4.62
4	0.061	11.35	22.06	16.73	31.81	126.54	59.78	35.41	12.67
5	0.180	3.85	27.73	34.68	43.7	66.4	28.84	79.84	4.63
6	0.173	4.01	22.17	48.14	62.72	54.21	20.77	125.64	5.21
7	0.133	5.20	11.86	24.50	37.31	113.86	40.81	68.36	7.15
8	0.129	5.36	14.61	58.52	86.04	51.84	17.09	177.5	7.09
9	0.291	2.38	27.09	67.19	74.29	37.75	14.88	170.44	3.39
Mean	0.182	4.61	18.64	36.00	47.23	84.15	34.50	93.26	5.81
SD	0.068	2.71	7.132	18.12	22.19	35.45	16.03	53.40	2.93
CV	0.37	0.59	0.382	0.50	0.47	0.42	0.46	0.57	0.51
CV [%]	37.5	58.9	38.30	50.34	46.97	42.12	46.5	57.2	50.56

\*K<sub>el</sub> – elimination rate constant, t<sub>0.5kel</sub> – biological half-life, C<sub>max</sub> – maximum plasma concentration, AUC<sub>last</sub> – area under the plasma concentration-time curve up to the last measurable concentration, AUC<sub>inf</sub> – area under the plasma concentration-time curve from zero to infinity, V<sub>d</sub> – volume of distribution, CL – clearance, AUMC<sub>last</sub> – area under the first moment curve up to the last measurable concentration, MRT – mean residence time, SD – standard deviation, CV – coefficient of variation

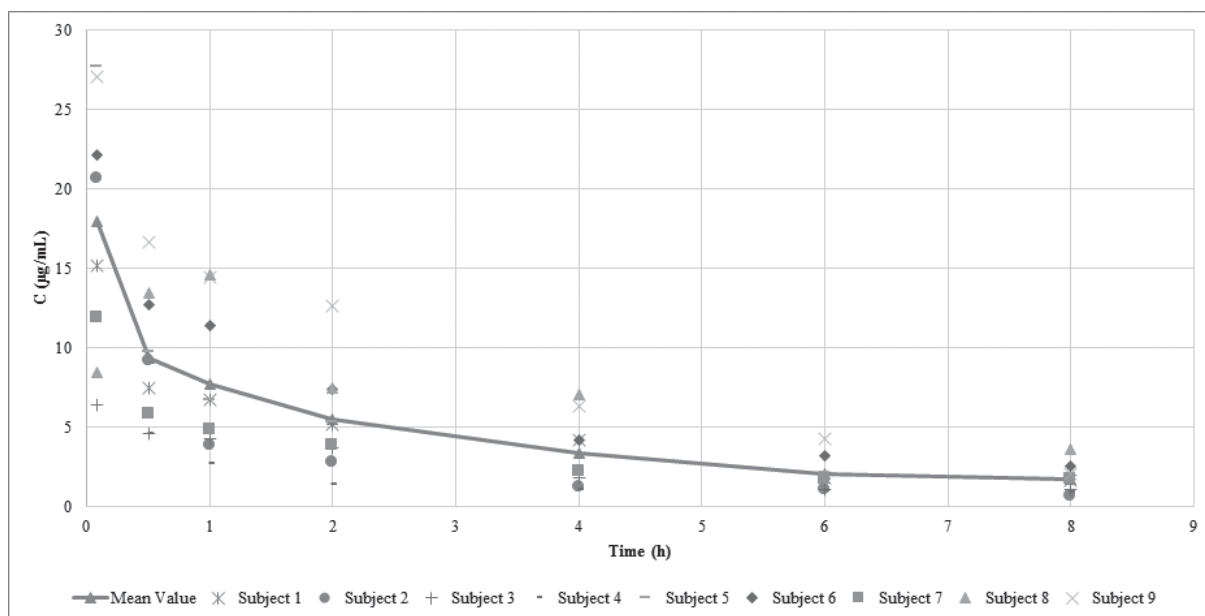


Figure 3. Concentrations [ $\mu\text{g}/\text{mL}$ ] of acetaminophen in the patients with pancreatitis ( $n = 9$ ) after a single *i.v.* dose of acetaminophen (1000 mg)

In the present study, there were 9 patients (4 women, 5 men) who received *i.v.* AAP (table VII). Patients were hospitalized at the Department of Gastroenterology in Gdańsk due to exacerbation of CP. Patients were in the age range of 27 to 67 years and weight range of 57.1 to 100.0 kg. All patients had relatively normal liver function except 2 alcoholic patients. ASPAT values were 41 U/I and 84 U/I (reference range 5–34 U/L) and ALAT was 64 U/I and 107 U/I (reference <55 U/L) [21]. 1 woman and 1 man were found to have a higher creatinine clearance than the normal range (normal range for females is 88–128 mL/min and for males 97–137 mL/min) [22], while 1 woman and 1 man were found to have lower creatinine clearance which was most likely caused by the age of the patients.

Figure 3 shows mean plasma concentration-time profiles for acetaminophen.

## Discussion

Our HPLC method was modified from the original method reported by Helmy et al [20]. In our method, we reduced the concentration of sodium dihydrogen phosphate buffer from 0.2 to 0.1 M. Moreover, pH increased in our method from 3 to 3.5 using phosphoric acid. The essential economic modification is decreasing

the injection volume to 2  $\mu\text{L}$ , while Helmy et al. used 25  $\mu\text{L}$ , which mean we reduced the parameter over ten times. Phenacetin was used in our method as internal standard compared to tinidazole. Slight difference in flow rate was used in our method 1 mL/min compared to 1.2 mL/min.

Our samples gave good results when stored at  $-22^{\circ}\text{C}$  until examination compared to  $-80^{\circ}\text{C}$ . In addition, the polytetrafluoroethylene was centrifuged at 7000 rpm for 6 min in room temperature, but Helmy et al. centrifuged it at 6000 rpm for 10 min at  $4^{\circ}\text{C}$ .

Our modified analytical method had various advantages such as using phenacetin, as an internal standard (IS), that improved accuracy and precision of the analysis. Concerning economic and bioethical reasons, the simple extraction procedure small plasma volume (350  $\mu\text{L}$ ) and small injection volume (2  $\mu\text{L}$ ), made the method reliable and useful in case of analysing expensive and rare samples. The calibration curves included all drug concentrations measured in clinical practice and acetaminophen concentrations from the therapeutic range (5–20  $\mu\text{g}/\text{mL}$ ). The within- and between-day accuracy and precision were in accordance with European Medicines Agency (EMA) guidelines [23].



The validation of the method included a wide range of plasma concentrations (1–30 µg/mL) and may be introduced as a proper method for PK studies, TDM (therapeutic drug monitoring) implementation, and conventional clinical applications. The authors used the method for measuring of plasma acetaminophen concentrations in patients with CP. Next, the pharmacokinetic parameters were calculated.

Comparing the obtained pharmacokinetic parameters between the analysed patients and the healthy volunteers (n=24) (Study 98051C-CIS) [24], who received the same *i.v.* dose of acetaminophen (1000 mg), the  $C_{max}$  for the group with CP was lower than values presented in healthy ones by 37.7%. The mean  $C_{max}$  value for acetaminophen for pancreatic patients was  $18.64 \pm 7.13$  µg/mL and for healthy volunteers it was  $29.9 \pm 8.3$  µg/mL.  $AUC_{0-t}$  showed much lower exposure in pancreatic patients. The values were  $36.00 \pm 18.12$  µg·h/mL for pancreatic patients and  $57.6 \pm 10.4$  µg·h/mL in the healthy volunteers [24]. Besides,  $t_{0.5}$  was almost doubled in the pancreatic patients compared to the healthy ones, from the values  $2.7 \pm 0.4$  h in healthy individuals, to  $4.61 \pm 2.71$  h in pancreatic patients.

$AUC_{0-t}$ ,  $C_{max}$ ,  $T_{max}$  and  $t_{0.5}$  were compared also with another study with volunteers (n=34) receiving the same *i.v.* dose of acetaminophen (Study CPI-APA-001) [24] and the results confirmed the differences between the parameters in pancreatic patients.  $AUC_{0-t}$

for healthy volunteers was  $42.3 \pm 10.58$  µg·h/mL and  $36.00 \pm 18.12$  µg·h/mL for pancreatic patients indicating a strong evidence of the lowering of  $AUC_{0-t}$  in pancreatitis.  $C_{max}$  also showed decreased by 34.36% in pancreatic patients ( $18.64 \pm 7.13$  µg/mL compared with  $28.4 \pm 21.17$  µg/mL in healthy volunteers). Finally,  $t_{0.5}$  for pancreatic patients was much higher than healthy volunteers with values of  $4.61 \pm 2.71$  h and  $2.39 \pm 0.57$  h for both of subjects, respectively. It suggests longer elimination of the drug in chronic pancreatitis.

Finally, in our patients the CP had an influence on the concentrations of acetaminophen as compared with the healthy volunteers. This means that there is a need for dose modification in this group of patients. A sensitive and simple HPLC method for acetaminophen analysis in human plasma was validated and applied for the pharmacokinetic study of acetaminophen in the patients.

#### Conflict of interest

None

Correspondence address:

✉ Hanna Urjasz

Department of Clinical Pharmacy and Biopharmacy  
Medical University of Poznań

Marii Magdaleny 14, PL 61-861 Poznań, Poland

☎ (+48 61) 668 78 65

✉ hanna.urasz@gmail.com

#### References

1. National Digestive Diseases Information Clearinghouse NIH Publication, July 2008;08–1596.
2. Nair RJ, Lawrel LB, Miller MR. Chronic Pancreatitis. *Am Family Physician*. 2007;76(11):1679–88.
3. Warshaw AL, Banks PA, Fernandez-Del Castillo C. AGA technical review: treatment of pain in chronic pancreatitis. *Gastroenterology*. 1998;115:765–76.
4. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology*. 2013; 144(6):1252–61.
5. Lin Y, Tamakoshi A, Matsuno S, et al. Nationwide epidemiological survey of chronic pancreatitis in Japan. *J Gastroenterol*. 2000;35:136–41.
6. Chen WX, Zhang WF, Li B, et al. Clinical manifestations of patients with chronic pancreatitis. *Hepatobiliary Pancreat Dis Int*. 2006;5:133–7.
7. Tenner S, Baillie J, DeWitt J, et al. Management of Acute Pancreatitis. *Am J Gastroenterol* advance online publication 2013;108(9):1400–15.
8. Govt. of India, Ministry of Health and Family Welfare. Vol. 2. Delhi: Publication by Controller of Publication; 1996. *Indian Pharmacopoeia*; pp. 484–554.
9. Bonnefont J, Courade JP, Alloui A, et al. Antinociceptive mechanism of action of paracetamol. *Drugs*. 2003; 63: 1–4.



10. Vane JR, Botting RM. The history of anti-inflammatory drugs and their mechanism of action. In: Bazan N, Botting J, Vane J, editors. *New targets in inflammation: inhibitors of COX-2 or adhesion molecules*. Dordrecht: Kluwer Academic; 1996. p. 1-12.
11. AFT Pharmaceuticals Pty Ltd. *PARACETAMOL-AFT*. 2013;6: 3.
12. Forrest JA, Clements JA, Prescott LF. Clinical Pharmacokinetics of Paracetamol. *Clin Pharmacokinet*. 1982;7(2):93-107.
13. Burton M, Shaw L, Schentag J, Evans W. *Applied Pharmacokinetics & Pharmacodynamics: Principles of Therapeutic Drug Monitoring* 2006;4:754.
14. Hinchman CA, Nazzareno B. Glutathione Conjugation and Conversion to Mercapturic Acids Can Occur as an Intrahepatic Process. *J Toxicol Environ Health*. 1994;41(4):387-409.
15. Peery AE, Dellon ES, Lund J, et al. Burden of gastrointestinal diseases in the United States: 2012 Update. *Gastroenterology*. 2012;143:1179-87.
16. Fagenholz PJ, Fernandez-del Castillo C, Harris NS, et al. Direct medical costs of acute pancreatitis hospitalizations in the United States. *Pancreas*. 2007;35:302-7.
17. Olesen AE, Brokjaer A, Fisher I, et al. Pharmacological challenges in chronic pancreatitis. *World J Gastroenterol*. 2013;19(42):7302-7.
18. Hammer HF. Pancreatic exocrine insufficiency: diagnostic evaluation and replacement therapy with pancreatic enzymes. *Dig Dis*. 2010;28(2):339-43.
19. Forsmark CE. Chronic pancreatitis and malabsorption. *Am J Gastroenterol*. 2004;99(7):1355-7.
20. Helmy SA, El-Bedaiwy HM. Simultaneous Determination of Paracetamol and Methocarbamol in Human Plasma By HPLC Using UV Detection with Time Programming: Application to pharmacokinetic Study. *Drug Res*. 2014;64:363-7.
21. Paparoupa M, Pietrzak S, Gillissen A. Case Report Acute Rhabdomyolysis Associated with Coadministration of Levofloxacin and Simvastatin in a Patient with Normal Renal Function. *Case Rep Med*. 2014;2014:1-4.
22. Mythen M, Burdett E, Stephens R. *Assessment of Renal Function: Radiological*. Anaesthesiology: Churchill's Ready Reference, Elsevier Health Sciences, 2010;106-107.
23. European Medicine Agency: science medicines health Guideline on bioanalytical method validation, 21 July 2011 EMEA/CHMP/EWP/192217/2009 Rev.1 Corr. Committee for Medicinal Products for Human Use (CHMP). Retrieved Januray 8, 2017, from [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/\\_2011/08/WC500109686.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/_2011/08/WC500109686.pdf).
24. Ji, Suresh D, Yaning W. *Clinical Pharmacology and Biopharmaceutics Review(S)* Center For Drug Evaluation and Research. 2009; 022450Orig1s000: 4.