Acetaminophen Analysis in Different Commercial Formulation of Iranian Acetaminophen Tablets

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Abstract

Acetaminophen is one of the most important antipyretic and analgesic drugs. It has an excellent efficacy when it is used in therapeutic doses, but in unsafe doses it can be resulted in hepatotoxicity and permanent liver failure. Due to disparities that have been reported between the actual and stated concentration of acetaminophen tablets, we aimed to compare the actual quantity of 8 Iranian generic acetaminophen tablets with the stated amount on the label, using HPLC method. Drug concentration has been measured by HPLC. We performed USP procedure for all models such as tablets, capsule, and oral solution assay preparations USP-36 NF31 by standard preparations. Method validation was achieved. For USP guidelines performance we need to concern on standard references. By chromatography equipment, we used HPLC analyzer 1200 degasser, 1200 bin pump, 1200 ALS, and 1200 VWD. Acetaminophen sample solution included 325 mg weighted quantity of the powder that transferred to a 200 ml volumetric flask in addition to 100 ml of mobile phase. A part of this solution was transferred throughout a 0.5 micrometer permeable filter (or finer). By injecting 10 μL of standard solution into the chromatograph, major peaks are measured as a response. As a final point, calculation of the quantity of acetaminophen in each brand was obtained via specific formula. According to the USP, all brands consisted of 90.0– 110.0 % of the labeled amount of active ingredient ($p < 0.05$). Consequently, The USP standards are met for drugs with different lot numbers by a variety of companies. The differences of clinical attributes of Paracetamol overdose between Iran and other countries may be related to pharmacokinetic and pharmacodynamics issues, metabolism, genetic factors or environmental effects. Further studies are recommended.

Keywords: Acetaminophen, Analysis, Generic tablets, HPLC, Paracetamol overdose, Quality control

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1. Introduction

Acetaminophen, an active metabolite of acetanilide and phenacetin, also known as N-acetyl-p-aminophenol, APAP, and paracetamol, is a popular painkiller and antipyretic medications. The main factor leading to overdose and overdose-related liver dysfunction and death in disparate countries [1, 2].Paracetamol is a white and bitter taste tablet which was discovered in 1893.From the pharmacokinetic point of view, the therapeutically amount for children is 10 to 15mg/kg per dose and 325 to 1000 mg per dose for adults. Usually, toxicity happens with single injection more than 250 mg/kg or those greater than 12 g over a 24-hour period [3]. From the biochemical aspect, having absorbed hastily and entirely from the duodenum, acetaminophen's metabolism takes place within the hepatic microsomes. Ninety percent of acetaminophen in the liver is transformed to sulfate and glucuronide conjugates which are urinated. The rest is metabolized via oxidation by the hepatic cytochrome P450 into NAPQI which is a toxic, electrophilic intermediated substance with high reactivity [4]. Production of a reactive metabolite by the cytochrome P450 system and protein formation can lead to disruption in mitochondria function and result in oxidant stress. Eventually, lead to necrotic cell death [5].

Today, High-Performance Liquid chromatography is the most common analytical technique, using for separation and analysis of mixtures [6]. HPLC, which developed in the 1960s, has been used for manufacturing pharmaceutical and biological products, research on the components of a complex biological/synthetic samples and medical intentions [7].

Recently, there are many arguments about the relative advantages and drawbacks of this issue in developing countries. Monitoring of the quality of generic forms is studied in few studies [8, 9] and some discrepancies have been reported between the actual and stated concentration of medications. The main objective of this work, therefore is to compare the actual amount of 8 Iranian generic acetaminophen tablets, determined by HPLC method, with the stated amount on the label.

2. Materials and Methods

Our experimental study protocol was reviewed and approved by research ethics committee behalf Toxicological Research Center (Research project number 444.1396), Shahid Beheshti University of Medical Sciences, Iran. Acetaminophen was obtained in October 2016 from pharmacies in Tehran, Iran. The remedies were acquired as other consumers would found them. The drugs were stored at room temperature in a safe location until analysis. Table 2 illustrates a summary of

the origin and batch number of tablets of the different brands.

2.1. Apparatus

The chromatographic system consisted of an Agilent 1200 (Germany Corporation) series chromatograph equipped with auto-sampler (G1329A), UV detector (G1314B), in built solvent Degasser (G1379), Binary pump (G1312A), and Chem Station software Rev.B.03.0. The analytical column was C8-3 (5μm, 150 × 4.6 mm).

2.2. Reagents and Standards

All chemicals used were of analytical and HPLC grade including acetonitrile (Merck, KGaA, Germany). The distilled water filtered through 0.45 μm filter (Polypropylene) was used to prepare solutions. USP reference standards were used to ensure the procedures met USP guidelines for precision, chromatographic tailing, theoretical plates, and comparison between the drugs being analyzed and the internal standard. Drug concentration was determined using method described by USP-36 NF31 for assay of tablets. Method validation was achieved for each drug prior to analysis. In the Quality control section, the USP standards were checked to meet the established amount for the analysis. After that, the actual and stated concentrations for each drug purchased inform the market was compared.

2.3. Assay in Dosage Forms

In order to provide the acetaminophen sample solution, twenty tablets were weighed, powdered and then a precisely weighted quantity of the powder, equivalent to 325 mg of acetaminophen, transferred to a 200 ml volumetric flask. In the next step 100 ml of mobile phase was added and finally, the solution was shaken for 10 minutes by mechanical mixer. After that, this solution was sonicated for 5 minutes, then diluted using mobile phase and gently mixed. A portion of this solution was transferred throughout a 0.5-micrometer porosity filter (or finer) and the filter first 10 mL of this filtrated solution was discarded. 10 µl of the standard solution, as well as samples of different acetaminophen tablets produced by the companies evaluated, were injected to the chromatography column and the major peaks were recorded. Finally, the quantity of acetaminophen in each brand was obtained.

2.4. Statistical Analysis

All analyses were carried out using SPSS, version 22 to compare the content of various generics with the standard. At 95% confidence interval, $p \leq 0.05$ was considered significant.

3. Results and Discussion

The analytical method for analyzing each sample by chromatographic columns, mobile phases, flow rates, and detector wavelengths are listed in Table 1. In order to prepare a standard solution with a concentration of 0.01 mg/ml we dissolved a precise quantity of 325 mg acetaminophen. The samples and standard's peaks were obtained which illustrated in figure 1. The mean percent of the labeled amount of each sample produced in

Table 1. High-performance liquid chromatographic (HPLC) analysis of Iran medication production.

| Drug	HPLC Column (mm)	Mobile Phase (vol /vol)	Flow Rate (ml/min)	Wavelength(nm)	Retention time (min)	CV %
A					7.06	4.303
B					7.130	6.874
C					7.153	0.001
D					7.245	0.001
E	L_1 (C_{18})	Degassed water			7.217	0.001
F	3.9 mm $\times$ 30	and methanol	1	243	7.190	0.002
G	cm	(3:1)			7.217	1.841
H					7.34	0.001

Table 2. Results of assays for eight acetaminophen brands in Iran.

Company	Area I	Area II	Area III	Mean	SD	RSD%	10,000 C (r_u / r_s)
A	1371.6	1370.7	1370.5	1370.933	0.59	0.04	320.75
B	1381.0	1382.1	1382.9	1382.0	0.95	0.07	323.34
C	1383.7	1380.5	1384.3	1382.833	2.04	0.15	323.53
D	1391.1	1389.2	1385.9	1388.733	2.63	0.19	324.91
E	1387.6	1389.2	1391.0	1389.266	1.70	0.12	325.04
F	1397.1	1392	1396.8	1395.3	2.86	0.21	326.45
G	1357.9	1357.6	1358.1	1357.866	0.25	0.02	317.69
H	1393.5	1396.6	1397.1	1395.733	1.95	0.14	326.55
Standard	1388.1	1388.9	1390.3	1389.1	1.11	0.08	325.00

Iran were within the percent of labeled amount the ranges of USP-defined standards. According to the USP, all brands consisted of 90.0– 110.0 % of the labeled amount of active ingredient (p<0.05). Therefore, the USP standards have been met for drugs with different lot numbers from different types of companies. (Table 2)

Acetaminophen in therapeutic doses is a safe medicine, but in overdose, it can cause a severe adverse effect like hepatic necrosis and failure, as a predominant complication, renal, pancreatic, and cardiac failure [10]. The puzzling condition of acetaminophen toxicity has been varying between Iranian population and other countries worldwide. Whereas, high

dosage of acetaminophen intake was observed in our patients, the toxicity level in the majority of cases was low. However, we decided to compare the active ingredient of Iranian generic acetaminophen with USP standards. According to a high level of toxicity with immense amounts of acetaminophen (150-300 tablets) that have been observed in suicidal patients referring to the Loghman-Hakim Hospital, a unique referral center for toxic patients placed in Tehran, Iran. We planned to design a study that we have intended to compare the acetaminophen ingredient in our market's tablet with the USP standard dosage. Based on our retrospective data surveys in this referral center, we
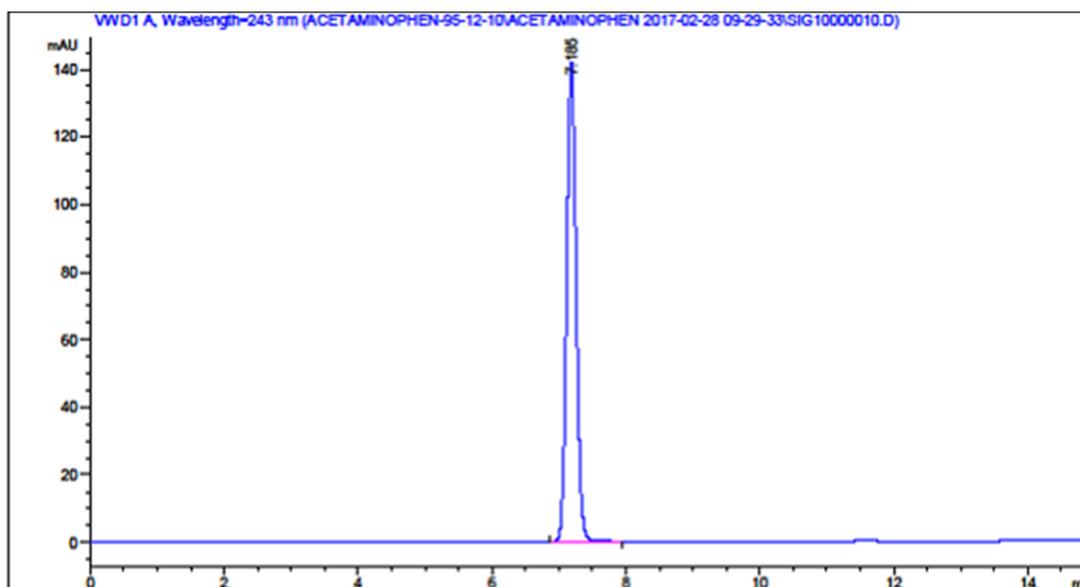
Figure 1. Standard's peaks of Paracetamol.

demonstrated two groups of patients using a maximum of 120 tablets (500 mg) in each group. Depending on the time elapsed between drug consumption and hospital admission, we had different toxic complications. In addition, according to two references (Meds cape [11] and Up-to-date [12, 13]), the toxic level of serum acetaminophen must be greater than 200 mcg/ml. Besides, founded on the Gold frank's Toxicological Emergencies reference book, a toxic point of serum acetaminophen has to be greater than 150 mcg/ml. Consequently, the preceding information caused the puzzling state of acetaminophen toxicity.

Although absorption of acetaminophen subsequent to oral therapeutics dose is rapid, absorption by dose increase may be more slowly and bioavailability of acetaminophen has different range, from 70 to 90% [10].

Furthermore, Pharmacokinetics of acetaminophen taken in overdose may vary from those detected following therapeutic doses. These Differences are as a result of both dose-dependent variations and the effects of drugs or pathological consequences of the overdose on kinetics.

The small intestine is the dominant site of acetaminophen absorption. The rate of drug absorption relies upon its dissolution and gastric emptying, which may regulate transit time into the small intestine [14].

On the other hand, final drug absorption relies on its solubility and the time of drug that remains in the small intestine. An over dosage of a drug causes a delay in its absorption, and also onset or peak of its action. The responsible mechanism for this fact includes the formation of a poorly soluble mass of drug, and a decrease in gastrointestinal motility which bezoars mass subsequent of mechanical obstruction in the small intestine [14, 15].

Another reason of less toxicity and complication in our patients is probably

genetic variation. There is some evidence that genetic mutation in some human enzymes such as glutathione transferase may affect morbidity and mortality of acetaminophen toxicity [10]. Actually, Polymorphisms in the cytochrome isoenzymes [16, 17] ,variation in other enzymes which involved acetaminophen metabolism, such as UDP-Glucuronosyltransferase (UGT), sulfotransferases (SULT), glutathione S-transferase (GST), N-deacetylase, N-acetyltransferase-2 (NAT2), and fatty acid amide hydrolase can lead to increase or decrease oxidative metabolism and toxicity of acetaminophen [18].

Contrary to our expectations, this study did not find a significant difference between Iranian generic acetaminophen and USP standard by HPLC method but reported toxicity in patients was less than expected. Although Acetaminophen is one of the most commonly agents of hepatotoxicity around the world, it is not a life threatening drug in Iran. This study was carried out for the first time in Iran. The limitations of our study were as follows: first, we selected only 8 brands of paracetamol tablets out of 40 different commercial brands to analyze in this study. Due to this, we cannot generalize our results to other brands. Secondly, we were not able to compare the Iranian Paracetamol tablet to its universal exemplary. Finally, the survey on experimental aspect of Acetaminophen has been accomplished, but the more accurate analysis and resolution will be completed by future study of mine as characterizing the paracetamol metabolism in patients with pure

acetaminophen toxicity in Loghman-Hakim Hospital, Tehran, Iran, 2017.

4. Conclusion

In conclusion, our study demonstrates that the mean percent of all the various Iranian manufactured samples within the USP-defined are within acceptable ranges of the labeled amount. Hence, the differences in clinical characteristics of acetaminophen overdose between Iranian and other countries may be related to pharmacokinetic and pharmacodynamics of acetaminophen, Paracetamol metabolism, genetic factors or environmental effects like diet, the particle size of drug, and difference of drug solubility in the small intestine. Due to this disparity, we should investigate the probable causes as previously mentioned in Iranian population owing to high consumption of analgesics especially acetaminophen in further studies.

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References

[1] Schilling A, Corey R, Leonard M, Eghtesad B. Acetaminophen: old drug, new warnings. *Cleve. Clin. J. Med.* (2010) 77(1): 19-27.

[2] Chun LJ, Tong MJ, Busuttil RW, Hiatt JR. Acetaminophen hepatotoxicity and acute liver failure. *J. Clin. Gastroenterol.* (2009) 43(4): 342-349.

[3] Lewis RK, Paloucek FP. Assessment and treatment of acetaminophen overdose. *Clin. pharm.* (1991) 10(10): 765-774. PMID: 1683827.

[4] McGill MR, Jaeschke H. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. *J. Pharm. Res*. (2013) 30(9): 2174-2187. PMID: 23462933.

[5] Jiang J, Briede JJ, Jennen DG, Van Summeren A, Saritas-Brauers K, Schaart G, et al. Increased mitochondrial ROS formation by acetaminophen in human hepatic cells is associated with gene expression changes suggesting disruption of the mitochondrial electron transport chain. *Toxicol. Lett.* (2015) 234(2):139-150. PMID: 25704631.

[6] Gilbert MT. *High- performance liquid chromatography*, 1ST ed., Elsevier: Butterworth-Heinemann (2013).

[7] Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C, Spoendlin C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3μm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. *J. Chromatogr. A*. (2004) 1036(2): 127-133.

[8] Davignon JP, Trissel LA, Kleinman LM. Pharmaceutical assessment of amygdalin (Laetrile) products. *Cancer. Treat. Rep*. (1978) 62(1):99-104. PMID: 627001.

[9] Schwertner HA, Storrow AB. Comparison of actual and stated concentrations of pharmaceuticals manufactured in Mexico. *Clin. Ther*. (2005) 27(5): 609-615. PMID: 15978310.

[10] Josephy PD. Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology. *Hum. Genomics. Proteomics*. (2010) 2(1). PMID: 20981235

[11] Jones CM, Mack KA, Paulozzi LJ. Pharmaceutical overdose deaths, united states, 2010. *JAMA*. (2013) 309(7): 657-659. PMID: 23423407

[12] Lewis RK, Paloucek FP. Assessment and treatment of acetaminophen. *Clin. pharm*. (1991) 10: 765-774.

[13] McGill MR, Jaeschke H. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. *J. Pharm. Res*. (2013) 30(9): 2174-2187.

[14] Rosenberg J, Benowitz NL, Pond S. Pharmacokinetics of a drug overdose. *Clin. pharmacokinet*. (1981) 6(3): 161-192.

[15] Forrest JA, Clements J, Prescott L. Clinical pharmacokinetics of paracetamol. *Clin. pharmacokinet*. (1982) 7(2): 93-107.

[16] Ueshima Y, Tsutsumi M, Takase S, Matsuda Y, Kawahara H. Acetaminophen Metabolism in Patients with Different Cytochrome P-4502E1 Genotypes. *Alcohol. Clin. Exp. Res*. (1996) 20(1suppl): 25a-28a. PMID: 8659683

[17] Critchley J, Nimmo G, Gregson C, Woolhouse N, Prescott L. Inter-subject and ethnic differences in paracetamol metabolism. *Br. J. Clin. Pharmacol*. (1986) 22(6): 649-57.

[18] Zhao L, Pickering G. Paracetamol metabolism, and related genetic differences. *Drug. Metab. Rev*. (2011) 43(1): 41-52.

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