SIMULTANEOUS DETERMINATION OF PARACETAMOL AND ITS MAIN DEGRADATION PRODUCT IN GENERIC PARACETAMOL TABLETS USING REVERSE-PHASE HPLC

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ABSTRACT: A simple isocratic reversed-phase HPLC method was developed and validated for the determination of paracetamol and *p*-aminophenol, its major degradation impurity, in the pharmaceutical tablets. The analytes were separated on a C18 Inertsil® ODS-3 column (250 mm x 4.60 mm i.d., 5 μm particle size). A mobile phase, MeOH: 0.01M phosphate buffer pH 5.0 (30:70 v/v) at flow rate of 1 ml/min was suitable for the separation and determination of paracetamol and *p*-aminophenol. The UV detection was carried out at 243 nm. The chromatographic parameters such as retention times, capacity factor, tailing factor, number of theoretical plates, %RSD of peak area and resolution factors were determined. The developed method was found to be linear over concentration ranges of 2.5 - 20 μg/ml (r^2 = 0.9992 and 0.9997 for paracetamol and *p*-aminophenol, respectively). The limit of detection (LOD) for paracetamol and *p*-aminophenol were 0.06 and 0.5 μg/ml, respectively. The limit of quantification (LOQ) for paracetamol and *p*-aminophenol were 0.75 and 1.0 μg/ml, respectively. Validation acceptance criteria were met in all cases. The developed method was successfully applied to determine paracetamol and *p*-aminophenol in five generic paracetamol tablets in Thailand.

Keywords: Paracetamol, *p*-Aminophenol, HPLC

INTRODUCTION: Paracetamol is widely used as analgesic drug. It can be formulated in a variety of dosage forms e.g. tablets, syrups and suspensions, both in the single-ingredient and multi-component ones. It is accepted as a very effective treatment for the relief of pain and fever in a variety of patients including children, pregnant women, and the elderly[1]. Paracetamol is a *p*-aminophenol derivative, which synthesized by acetylation of *p*-aminophenol and acetic anhydride. It may be hydrolyzed to *p*-aminophenol in some conditions such as high temperature, acidic or basic media. *p*-Aminophenol is the main impurity in paracetamol preparations that may be formed during the storage of preparation or may be originated during the synthesis of paracetamol[2]. It was reported that *p*-aminophenol may cause nephrotoxicity and teratogenicity; therefore, its amount should be strictly controlled[3]. The United States Pharmacopeias (USP) and British Pharmacopeias (BP) limit the amount of *p*-aminophenol in paracetamol substance at 0.005% w/w. The limits of *p*-aminophenol may vary in different products depending on the dosage forms and formulations. The monograph of paracetamol tablets in BP, *p*-aminophenol is limited to 0.1% w/w, while no impurity testing method is presented in USP31[4,5]. The BP uses a spectrophotometric assay method for the determination of paracetamol tablets. The USP31 recommends method for paracetamol determination using HPLC method. All of these methods determine the amount of paracetamol only, none of them includes degradation product determination.

This research reports a rapid and sensitive HPLC determination method with UV detection, useful for routine quality control of paracetamol and its main degradation product, *p*-aminophenol in paracetamol tablets. The method was validated by parameters such as specificity, linearity, accuracy, and precision. The validated method was applied to 5 generic paracetamol tablets in Thailand for determination of paracetamol and *p*-aminophenol.

MATERIALS AND METHODS:

Apparatus and reagents

The analysis was performed on a HPLC system equipped a model series P1500 Pump, AS300 autosampler SpectraSYSTEM® and a UV 1000 SpectraSYMTEM®detector (Thermo Seperation Product, USA). A reversed-phase C18 column

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(Inertsil® ODS-3, 250 mm x 4.60 mm i.d., 5 µm particle size, Japan) was used for separation. The UV detection was carried out at 243 nm. Methanol (Labscan®, Ireland) was HPLC grade. Disodium hydrogen orthophosphate was supplied by Univar®, Australia in analytical grade. Paracetamol and *p*-aminophenol reference standard were purchased from Sigma Aldrich®, Germany.

Chromatographic condition

Chromatographic separation was achieved using reverse phase chromatography with isocratic elution. The mobile phase consisted of methanol and 0.01 M disodium hydrogen orthophosphate buffer pH 5 (30:70, v/v) and pumped at a flow rate 1.0 ml/min.

Preparation of the standard solutions

The stock solution containing 1 mg/ml of paracetamol and *p*-aminophenol was prepared in mobile phase. This solution was adequately diluted to determine the accuracy, precision, linearity and limits of detection and quantification.

Preparation of samples

For the assay, an average weight of 20 tablets was determined. The tablets were powdered and an accurate weight of the finely powdered equivalent to 50 mg of paracetamol was transferred into 50 ml volumetric flasks. Then 20 ml of mobile phase was added and the solution was mechanically shaken for 20 min. The flasks were filled with mobile phase. Thus, prepared solutions were filtered through filters (0.45 µm, Whatman, USA) and the first 5 ml of the filtrate was discarded. The filtrates were 100-fold diluted with mobile phase and 20 µl of the prepared solutions were injected into the column.

For the determination of *p*-aminophenol, an amount equivalent to 1,000 mg of paracetamol was suspended in 50 ml volumetric flasks and followed the same process as the assay preparations. Then, undiluted solution was used for determination.

RESULTS AND DISCUSSION:

Optimization of HPLC condition

The method optimization was carried out using the different pH ranges and polarity of mobile phase. The use of MeOH:H_2O (25:75) as in USP31 gave the asymmetry peak with tailing for *p*-aminophenol peak. Using 0.01M phosphate buffer with pH in range of 3.0-5.0 as aqueous phase, the peaks were well separated but the increasing of aqueous phase acidity, peaks were eluted close to solvent front, and therefore pH 5.0 was found to be optimal. Mobile phases of various composition of MeOH and 0.01 M phosphate buffer pH 5.0 were also investigated. With decreasing the polarity of mobile phase, the peak retention times were decreased. The best result was obtained using the mobile phase MeOH: 0.01M phosphate buffer pH 5.0 (30:70 v/v).

After optimization, HPLC method was carried out on Inersil® C18 CDS-3, 250 mm x 4.60 mm, 5 µm column using MeOH: 0.01M phosphate buffer pH 5.0 (30:70 v/v) as mobile phase at flow rate of 1 ml/min. The UV wavelength at 243 nm was chosen for monitoring the separation. Typical chromatogram is illustrated in Figure 1A. The retention times were 3.39 min for *p*-aminophenol and 5.98 min for paracetamol.

System suitability

System suitability was performed by using 10 µg/ml of *p*-aminophenol and paracetamol and evaluated by making six replicate injections. Chromatographic parameters calculated from experimental data, such as capacity factors (k'), tailing factors, number of theoretical plates, % RSD of peak area and resolution factors (R_s) are given in Table 1. The system was deemed to be suitable for use if the capacity factors were in the range of 2-20 ($2 < k' < 20$), lower than 2 for tailing factor, more than 2 for resolution ($R_s > 2$), greater than 2000 number of theoretical plates (N), resolution between paracetamol and *p*-amino-phenol of at least two and less than 2% relative standard deviation (%RSD) for peak area.

Method validation

a. Specificity

Specificity of a method can be defined as absence of any interference at retention times of samples. As lack of information in the composition of generic formulations, makes it difficult to assess selectivity by traditional analysis comparison with a placebo solution. The specificity of the method was demonstrated by injection of standard solution of paracetamol and

p-aminophenol at concentration of 5 μg/ml. The elution peaks of paracetamol and *p*-aminophenol were presented in representative chromatograms shown in Figure 1A with the retention times of 5.98 and 3.39, respectively.

b. Linearity

The linearity of paracetamol and *p*-aminophenol were studies by preparing standard solution at five different concentrations ranging from 2.5-20 μg/ml. Each concentration was injected in five replicates and the mean value of peak area was taken for the calibration curve. The calibration curve can be described using the following equations: y = 101064x - 37981, r^2 = 0.9992 for paracetamol and y = 29362x + 1172.1, r^2 = 0.9997 for *p*-aminophenol, where y is the peak area and x is the concentration in μg/ml.

c. Precision

The precision of the assay was studied with respect to both intra-day (repeatability) and inter-day (intermediated) precisions. Repeatability was calculated from five replicate injections of three different concentrations of paracetamol and *p*-aminophenol in the same equipment on the same day. Inter-day precision was checked with the same concentrations as intra-day assay and the determination of each compound was repeated day by day during three days. The results are given in Table 2. The intra-day precision (%RSD) for paracetamol and *p*-aminophenol were lower than 1.81% and 3.15%, respectively. The inter-day precision (%RSD) for paracetamol and *p*-aminophenol were lower than 2.82% and 4.17%, respectively.

d. Accuracy

The accuracy of method was confirmed by determination of paracetamol and *p*-aminophenol at concentrations of 7.5 and 18.0 μg/ml. The %recovery and SD for paracetamol were found to be 103.16±1.35% at 7.5 μg/ml and 100.41±0.90% at 18.0 μg/ml, for *p*-aminophenol 103.13±1.92% and 100.3±1.34% at concentration 7.5 and 18.0 μg/ml, respectively. (Table 3)

e. Limits of detection and quantification

Limit of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratio of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined calculating the signal-to-noise ratio for each compound by injecting a series of solution until the S/N ratio 3 for LOD and 10 for LOQ. LOD was found to be 0.06 and 0.5 μg/ml for paracetamol and *p*-aminophenol, respectively. LOQ was found to be 0.75 and 1.0 μg/ml for paracetamol and *p*-aminophenol, respectively.

Assay of paracetamol tablets

The developed method was applied for the analysis of five generic paracetamol tablets in Thailand. Representative chromatogram of assay paracetamol is shown (Figure 1B). It is interestingly that the percent of the labeled amount (%LA) of paracetamol in all products was always between 94 and 97% (Table 4). However, USP31 stated that paracetamol tablet contain not less than 90% and not more than 110% of labeled amount of paracetamol. The content of *p*-aminophenol in all five generic paracetamol tablets was found less than LOD; therefore, the amount of *p*-aminophenol could be negligible.

Table 1 System suitability data (n = 6)

Parameters	Paracetamol	*p*-Aminophenol
Capacity factor (k')	10.41 ± 0.36	5.81 ± 0.03
Tailing factor	1.140 ± 0.08	1.178 ± 0.13
Plate number (N)	7404 ± 0.23	4407 ± 0.03
% RSD of peak area	0.04	0.11
Resolution (R_s)	10.67 ± 0.08	

Table 2 Precision of HPLC method

Compounds	Conc. (μg/mL)	Precision (%RSD)	
		Intra-day*	Inter-day**
Paracetamol	2.48	1.81	2.82
	9.62	0.04	0.23
	19.68	0.78	1.31
p-Aminophenol	2.52	3.15	4.17
	9.57	0.12	0.28
	19.89	0.63	2.34

*n=5, **n=3

Table 3 Recovery of paracetamol and *p*-aminophenol)

Compounds	Conc. (μg/mL)	%Recovery* (mean ± SD)
Paracetamol	7.53	103.16 ± 1.35
	18.07	100.41 ± 0.90
p-Aminophenol	7.20	103.13 ± 1.92
	17.28	100.30 ± 1.34

*n=3

Table 4 Analysis of 5 generic paracetamol tablets

Sample	% LA of paracetamol (mean ± SD)	*p*-Aminophenol (mg)
A	96.11 ± 1.17	ND*
B	96.57 ± 0.53	ND*
C	97.31 ± 2.91	ND*
D	97.68 ± 1.10	ND*
E	94.98 ± 2.22	ND*

*ND = Not detected

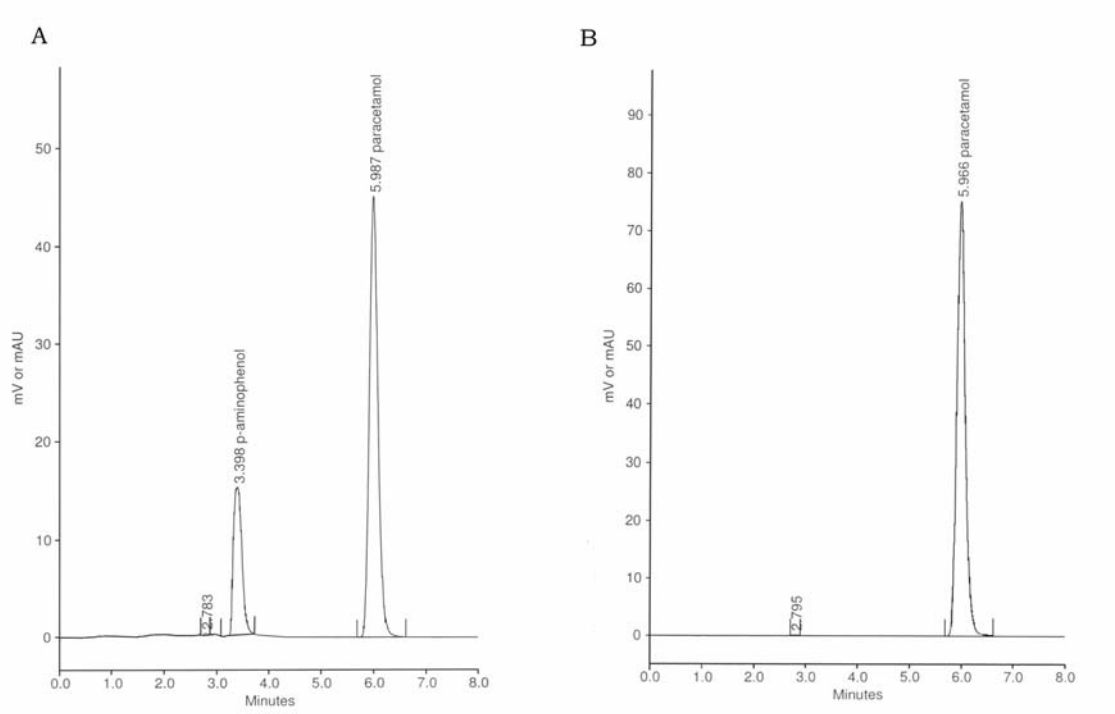

Figure 1 A) Chromatogram of standard paracetamol and *p*-aminophenol at concentration 5 μg/ml and B) Chromatogram of the assay paracetamol tablet

CONCLUSION: A simple and efficient reverse-phase HPLC method was found to be accurate, precise, and linear across the analytical range. The method was specific for the determination of paracetamol and *p*-aminophenol, its main degradation impurity. No significant amounts of *p*-amino-phenol were found in all generic paracetamol tablets. The developed method could be used for the determination of paracetamol and *p*-amino-phenol as its main degradation product in paracetamol tablet and could be applied on other dosage forms.

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REFERENCES:

1. Ward B, and Alexander-Williams JM. 1999. Paracetamol revisited: A review of the pharmacokinetics and pharmacodynamics. Acute Pain 2 (3): 139-49.

2. Roy J. 2002. Pharmaceutical impurities; A mini review. AAPs PharmSciTech 3 (2): article 6.

3. Song H and Chen T S. 2001. *p*-Aminophenol-induced liver toxicity: Tentative evidence of a role for acetaminophen J Biochem Mol Toxicol 15 (1): 34-40.

4. British Pharmacopeia. 2008. Paracetamol tablets, Stationery Office, London, UK.

5. The United States Pharmacopeial Convention, United States Pharmacopeia 31. 2008. Acetaminophen, The United States Pharmacopeial Convention, Rockville, USA.