A Reverse Phase High Performance Liquid Chromatographic Procedure for the Determination of Acetaminophen and its Degradation Product 4-aminophenol in Aged Pharmaceutical Formulations

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ABSTRACT

A stability-indicating ion pairing reverse phase high-performance liquid chromatographic method for the simultaneous determination of acetaminophen and its degradation product 4-aminophenol in pharmaceutical formulations is described. The separation was achieved isocratically at ambient temperature, on a µ-Bondapack C-18 column (5 µm, 30 cm x 3.9 mm i.d.) utilizing a mobile phase containing 0.05M phosphate buffer, methanol, and hexane-1-sulfonic acid sodium salt (850 : 150 : 2g), the pH was adjusted to 3 with glacial acetic acid. The flow rate was 1.5 ml min^{-1} with UV-detection (245 nm). Complete resolution of acetaminophen from its degradation product 4-aminophenol and co-formulated excipients could be achieved. The proposed method was successfully applied in post-marketing stability monitoring of the commercial batches of different acetaminophen containing drug formulations: tablets, drops, syrups, and suppositories.

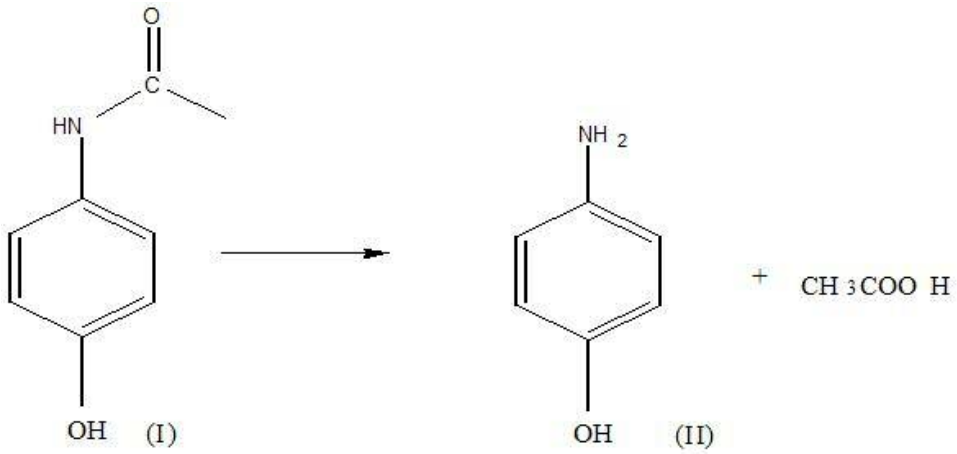
(Key words: acetaminophen, 4-aminophenol, determination, HPLC, stability-indication, ageing, post-marketing surveillance)

INTRODUCTION

Acetaminophen is the most widely used pharmaceutical analgesic and antipyretic agent throughout the world. It is contained in more than 100 drug products. Besides its use in common dosage forms such as tablets, capsules, syrups and suppositories, various children's chewable, suspension, and elixir formulations of acetaminophen are also available. Acetaminophen (I) is associated both with intentional and accidental poisoning and hepatotoxicity (Heubi *et al*. 1998; Farrell and Fernandez 2002). The assay and stability of acetaminophen preparations remained the topic of discussion by several workers (Connor *et al*. 1986). Direct UV measurement for several pharmaceutical formulations including acetaminophen formulations may be unsuitable due to interference of excipients and degradation products.

High-performance liquid chromatography is recognized to be a method of choice in pharmaceutical and biomedical analysis. The main route of acetaminophen (I) degradation is its hydrolysis to yield 4-aminophenol (II) and acetic acid (Scheme I).

Scheme I: Degradation of Acetaminophen (I) into 4-aminophenol (II).



The physical properties of acetaminophen tablets are affected by moisture. Factors such as temperature, humidity, and aging were found to affect the disintegration and dissolution pattern of acetaminophen tablets (Sarisuta and Parrott 1988). Bottom *et al*. (1997) investigated the dissolution pattern of acetaminophen capsules. Acetaminophen preparations were also found to be photosensitive (Mursyidi 1995; Waaler *et al*. 1997; Sinchaipanid *et al*. 1998). Some additives and antioxidants improved the chemical and physical stability of different acetaminophen formulations (Yan and Fan 1986).

Connor *et al*. (1986) reported the stability and acid or base catalysed hydrolysis of acetaminophen. The spectrophotometric methods reported for the determination of (I) and as an impurity for (II), are usually based on colour reactions (Korany *et al*., 1986; Mayadeo and Banavali, 1986; Milch and Szabo, 1991, Mohamed *et al*. 1997). Other methods include non-aqueous titration (Adelber and Frank, 1977), fluorometric (Street *et.al.*, 1979; Yoshida *et.al*., 1980), voltammetry (Shearer et al. 1972), and chromatographic analysis (Sisco *et al*. 1985; Suzuki *et.al*., 1990; Hewala 1994). Bergh and Lotter (1984) established a stability indicating gas liquid chromatographic method for the determination of acetaminophen in suppositories. High performance liquid chromatography (HPLC) was found to be a sensitive tool for the determination of (I) in the presence of other drugs (Ascah and Hunter 1988; El-Shanawany *et al*. 1991). Whelpton *et al.* (1993) made use of HPLC with dual electrode coulometric quantification in the redox mode for the determination of acetaminophen in blood while Liu *et al*. (1995) determined acetaminophen and its hydrolysis product by capillary electrophoresis with electrochemical detection. However, most of these methods are not suitable for simultaneous determination of (I) and (II) in the presence of preservatives, colorants, and flavors commonly added to liquid formulations.

The United States Pharmacopoeia 23 (1995) described a limit test for the detection of free 4-aminophenol (II) by UV-measurement in raw material, which is based on colour formation with alkaline nitroferricyanide solution. The USP Monograph of Acetaminophen oral solution, tablets, and suppositories described an HPLC method for acetaminophen determination without specifying any method or limit for the hydrolytic product (USP 1995). On the other hand, the HPLC method described for paracetamol (acetaminophen) and 4-aminophenol determination in oral solutions and suspensions by BP (1993) include detection at two different wavelengths.

The development of a simple stability-indicating HPLC method for the determination of acetaminophen and its degradation product in presence of common preservatives (benzoic acid or parabens), colouring agents, and flavours (caramel, raspberry red colour, etc.) in different dosage forms (syrups, drops, suspensions, suppositories, and tablets) would be an advantage. In this communication we wish to describe a reverse phase HPLC procedure capable of determining (I) and its degradation product (II) in different aged pharmaceutical formulations.

Table 1. Details of the Acetaminophen Containing Commercial Batches used in the Present Study.

S. No.	Brand Name	Manufacturer	Batch No.	Mfg. Date	Exp. Date
1.	Adol Syrup	Julphar, UAE	573	1/95	1/98
2.	Adol Syrup	Julphar, UAE	597	5/95	5/98
3.	Adol Syrup	Julphar, UAE	598	5/95	5/98
4.	Adol Syrup	Julphar, UAE	599	6/95	6/98
5.	Adol Syrup	Julphar, UAE	625	8/95	8/98
6.	Adol Syrup	Julphar, UAE	645	12/95	12/98
7.	Adol Syrup	Julphar, UAE	646	12/95	12/98
8.	Adol Syrup	Julphar, UAE	647	12/95	12/98
9.	Fevadol Syrup	Spimaco, K.S.A.	09974	9/94	9/97
10.	Fevadol Syrup	Spimaco, K.S.A.	11737	3/95	3/98
11.	Fevadol Syrup	Spimaco, K.S.A.	12744	7/95	7/98
12.	Fevadol Syrup	Spimaco, K.S.A.	13036	9/95	9/98
13.	Fevadol Syrup	Spimaco, K.S.A.	13041	11/95	11/98
14.	Fevadol Syrup	Spimaco, K.S.A.	14012	3/96	3/99

15.	Fevadol Syrup	Spimaco, K.S.A.	14432	4/96	4/99
16.	Fevadol Syrup	Spimaco, K.S.A.	14437	5/96	5/99
17.	Fevadol Syrup	Spimaco, K.S.A.	14738	6/96	6/99
18.	Adol Drops	Julphar, UAE	92	1/95	1/98
19.	Adol Drops	Julphar, UAE	94	3/95	3/98
20.	Adol Drops	Julphar, UAE	97	6/95	6/98
21.	Adol Drops	Julphar, UAE	99	9/95	9/98
22.	Adol Drops	Julphar, UAE	104	1/96	1/99
23.	Adol Suppositories	Julphar, UAE	37	6/94	6/97
24.	Fevadol Tablets	Spimaco, K.S.A.	18635	5/97	5/2000

UAE = United Arab Emirates.

K.S.A. = Kingdom of Saudi Arabia.

EXPERIMENTAL PROCEDURES

Materials

Acetaminophen and 4-aminophenol USP reference standards were used. Methanol (Fisons), hexane-1-sulfonic acid sodium salt were HPLC grade (Merck) and acetic acid (96%) was analytical grade (Merck).

Drug products: 17 batches of acetaminophen syrups belonging to 2 brands covering the shelf-life of the products, 5 batches of paediatric drops, 1 batch of suppositories, and 1 batch of tablets were collected from Riyadh (Saudi Arabia). The specifications are given in Table 1.

Distilled water. Bi-distilled in an all-glass still for all analytical purposes.

Instruments

The HPLC used was comprised of a Waters 600E System Processor, WISP 715 Autoinjector, Waters 991 Photodiode Array Detector, and PDA Integrator by Waters, Division of Millipore, Milford, MA (USA). A reverse phase C_{18} μ Bondapack 3.9 i.d. x 300 mm column was used. *Mobile phase*: The mobile phase consisted of 0.05M phosphate buffer : methanol : hexane-1-sulfonic acid sodium salt in the ratio of 850 : 150 : 2 (v/v/w), and the pH was adjusted to 3 with glacial acetic acid. 0.05M Phosphate buffer was prepared by dissolving 6.8 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in distilled water. It was

filtered through a membrane filter and degassing was carried out on-line by helium purging. The flow rate was 1.5 ml/min. *Detection.* UV (245 nm). The sensitivity was set at 0.25 AUFS and chart speed was 5 cm/min. and the column was maintained at ambient temperature. The autosampler was programmed to inject 20 µl of both the standards and samples.

Preparation of Different Solutions

1. Acetaminophen stock standard solution: 50 mg of acetaminophen USP reference standard was dissolved in 50 ml of methanol. 10 ml of this solution was diluted to 50 ml with the mobile phase to give a concentration of 200 µg/ml.

2. 4-Aminophenol stock standard solution: 50 mg of 4-aminophenol USP reference standard was dissolved in 50 ml of methanol. 5 ml of this solution was diluted to 50 ml with the mobile phase to give a concentration of 100 µg/ml.

3. Standard Solutions for Testing Linearity: Various concentrations of acetaminophen (I) were prepared by diluting the stock standard solution with mobile phase covering the range of 5 - 50 µg/ml and 50 - 100 µg/ml. For the hydrolytic product (II) various concentrations were prepared by diluting the stock standard solutions with mobile phase covering the ranges 0.5 - 5 µg/ml and 5 - 10 µg/ml.

4. Synthetic mixtures for testing accuracy and recovery: Synthetic mixtures containing (I) and its hydrolytic product (II) were prepared corresponding to varying levels of 4-aminophenol (0.1 - 2 µg/ml.).

5. Preparation of the Sample:

A. Syrups: 5 ml aliquot of the syrup or suspension (24 mg/ml or 120 mg/5 ml.) were diluted to 100 ml with methanol (Solution T)**.**

B. Drops: 1 ml aliquot of the drops (100 mg/ml.) were diluted to 100 ml with methanol. (Solution T).

C. Suppositories: Weighed 20 suppositories, and determined the average weight. They were cut into small pieces, weighed and transferred some pieces selected at random equivalent to 120 mg of acetaminophen into a 400 ml beaker, and then about 40 ml of 0.1 M. hydrochloric acid was added. It was gently warmed on a water bath, until melting of the base, with constant stirring. For cooling and solidification of the base the beaker was kept in refrigerator. The liquid was transferred to a 100 ml, volumetric flask through a funnel with a piece of cotton moistened with 0.1 M HCl. Repeated the procedure for total extraction and finally made up the volume to 100 ml, with the same solvent (Solution T).

D. Tablets: Weighed and powdered 20 tablets. From the powder a quantity equivalent to 120 mg of acetaminophen was taken in 60 ml of 0.1M HCl. After shaking for 20 min. it was filtered and 100 ml volume was made by adding 0.1M HCl (Solution T). For HPLC analysis, 1 ml, of solution T (from all the above samples) was transferred separately to 100 ml volumetric flasks and the volume was adjusted up to the mark with the mobile phase.

Assay Procedure

In each case 20 µl of standard and test were injected to HPLC. The concentration of each component injected was always within the linearity range.

RESULTS AND DISCUSSION

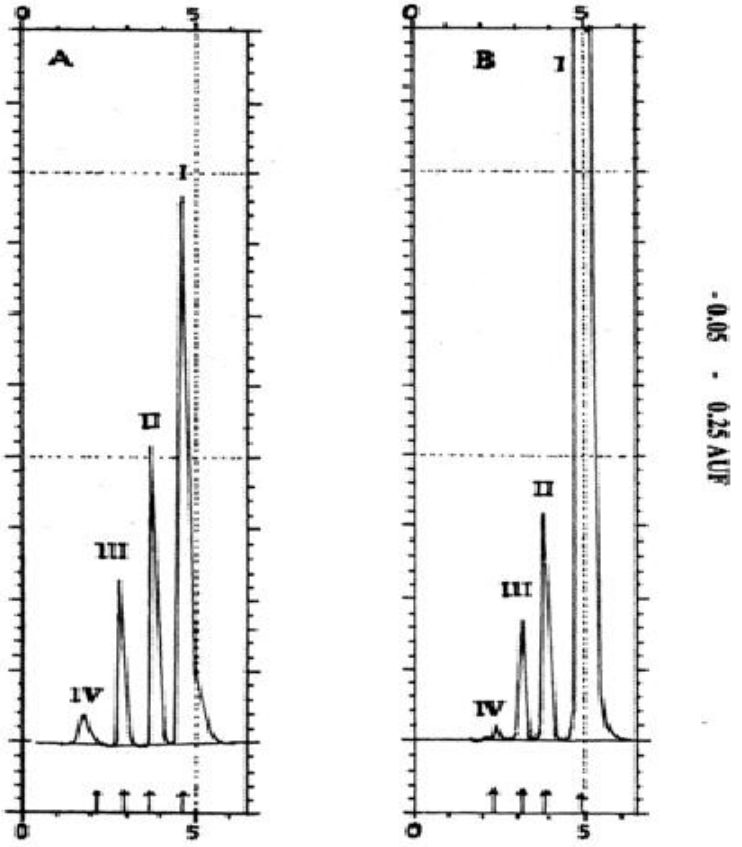
Development of Stability Indicating HPLC Assay Method

The chromatogram obtained by the proposed method (Figure 1) showed good separation of synthetic mixtures of acetaminophen (I) and its hydrolytic product 4-aminophenol (II). The retention time for (II) was 3.4 min. and for (I) 4.8 min. The relative retention times with respect to acetaminophen were 0.7 and 1.0 and the capacity factors were 5.8 and 8.6 for (II) and (I), respectively.

Figure 1. HPLC Resolution of:

A) A Synthetic Mixture of Acetaminophen (I), 4-aminophenol (II), Benzoic Acid (III) and Raspberry Red (IV)

B) A Commercial Acetaminophen Preparation Stored for 30 Months at Room Temperature.



Development and Validation of the Method

The proposed method was tested for linearity, reproducibility, selectivity, limit of detection and limit of quantitation for both components: (I) and (II). The applicability of the proposed HPLC method as stability-indicating was tested by using synthetic mixtures of (I) and (II) in different concentrations.

1. Precision: Reproducibility was assessed through calculation of the relative standard deviation (RSD%) of six replicates (n = 6) of each component (I and II) at two different concentrations. Acetaminophen (I) at a concentration of 20 µg/ml and 60 µg/ml showed RSD = 0.91 and 0.62 respectively. On the other side 4-aminophenol (II) at a concentration of 1 µg/ml and 5 µg/ml showed RSD = 1.35 and 0.98 respectively.

2. Linearity: The linearity tested for (I) over a concentration range of 5 - 50 µg/ml and 50 - 100 µg/ml showed the value of correlation coefficient "r" as 0.9998 and 0.9999, the slope 0.193, 0.0689 and the intercept 0.00289 and 0.000609, respectively. Upon linear regression analysis the equations obtained were: Y = 0.0193 X + 0.00289 and Y = 0.0689 X + 0.000609.

For 4-aminophenol (II) the linearity was maintained over the range 0.5 - 5.0 µg/ml and 5 - 10 µg/ml. The correlation coefficient "r" was 0.9999 and 0.9999, the slope 0.00293 and 0.000893, while the intercept was 0.00246 and 0.000464, respectively. Upon linear regression analysis the equations obtained were: Y = 0.00293 X + 0.00246 and Y = 0.000893 + 0.000464.

3. Sensitivity:

A: Limit of Detection (LOD): The limit of detection for (I) was 0.005 µg injected and for (II) 0.003 µg injected.

B: Limit of Quantitation (LOQ): The limit of quantification for (I) was found to be 0.001 µg injected and for (II) 0.005 µg injected.

4. Accuracy: The accuracy of the proposed method was determined by analysis of 6 synthetic mixtures containing varying levels of the hydrolytic product (II). The results obtained are presented in Table 2. The results clearly demonstrated that the suggested method is sufficiently sensitive for the determination of small concentrations of 4-aminophenol (0.1 µg/ml). The results are reproducible and precise as the relative standard deviations are within 0.3 and 1.9.

5. Selectivity: The selectivity (Figure 1) and applicability of the suggested method was verified by examining the post-marketing stability testing of some commercial acetaminophen containing formulations. The chromatograms obtained showed good resolution of all peaks and no interference due to the presence of inactive ingredients was observed. Based on these results it is concluded that the HPLC method developed in this study has the required sensitivity, selectivity, and reproducibility which made it versatile and perfect for the quantitative determination of acetaminophen and 4-aminophenol in different dosage forms.

Table 2. Result of Analysis by Suggested Method of Six Synthetic Mixtures Containing Acetaminophen and its Hydrolytic Product.

Component	Concentration added (µg/ml)	Mean % Recovery ± SE
Mixture-1		
Acetaminophen	25.0	100.5 ± 0.7
4-Aminophenol	2.0	100.7 ± 0.4
Mixture-2		
Acetaminophen	25.0	101.2 ± 0.3
4-Aminophenol	1.5	99.8 ± 0.9
Mixture-3		
Acetaminophen	25.0	99.7 ± 1.1
4-Aminophenol	1.0	101.6 ± 0.8
Mixture-4		
Acetaminophen	25.0	99.4 ± 0.6
4-Aminophenol	0.5	99.2 ± 0.7
Mixture-5		
Acetaminophen	25.0	100.2 ± 0.3
4-Aminophenol	0.25	99.1 ± 1.8
Mixture-6		
Acetaminophen	25.0	100.5 ± 0.5
4-Aminophenol	0.10	98.5 ± 1.6

* Mean of 4 experiments. RSD: Acetaminophen (0.3 - 1.1%), 4-Aminophenol (0.4 - 1.8%)

Table 3. Results of Analysis of Acetaminophen Containing Commercial Batches Used in the Present Study.

S.No.	Brand Name (Batch No.)	Age	pH	Acetaminophen (I) Assay % ± S.E.	4-Aminophenol (II) w/v ± S.E.
1.	Adol Syrup (573)	26	3.7	95.1 ± 1.21	3.20 ± 0.98
2.	Adol Syrup (597)	22	3.8	96.7 ± 0.75	3.10 ± 1.31
3.	Adol Syrup (598)	22	4.5	97.5 ± 1.31	1.08 ± 0.85
4.	Adol Syrup (599)	21	4.7	95.1 ± 0.78	1.15 ± 0.75
5.	Adol Syrup (625)	19	4.8	96.3 ± 0.80	1.10 ± 1.35
6.	Adol Syrup (645)	15	5.4	96.8 ± 0.90	0.80 ± 1.10
7.	Adol Syrup (646)	15	5.4	98.1 ± 0.25	0.70 ± 1.10
8.	Adol Syrup (647)	15	5.5	97.5 ± 0.81	0.90 ± 1.10
9.	Fevadol Syrup (09974)	30	3.3	95.8 ± 1.20	2.80 ± 0.90
10.	Fevadol Syrup (11737)	24	3.4	95.8 ± 0.80	2.50 ± 0.35
11.	Fevadol Syrup (12744)	20	3.4	96.1 ± 0.70	2.40 ± 0.40
12.	Fevadol Syrup (13036)	18	4.5	96.5 ± 0.60	1.80 ± 0.55
13.	Fevadol Syrup (13041)	16	4.8	95.8 ± 0.38	0.65 ± 0.61
14.	Fevadol Syrup (14012)	12	5.2	96.8 ± 0.31	0.50 ± 0.21
15.	Fevadol Syrup (14432)	11	5.5	97.2 ± 0.21	0.32 ± 0.40
16.	Fevadol Syrup (14437)	10	5.4	96.9 ± 0.21	0.30 ± 0.15
17.	Fevadol Syrup (14738)	9	5.6	97.5 ± 0.15	0.25 ± 0.10
18.	Adol Drops (92)	26	4.9	95.2 ± 1.01	3.25 ± 0.85
19.	Adol Drops (94)	24	5.2	96.2 ± 0.75	2.85 ± 1.25
20.	Adol Drops (97)	21	5.1	97.5 ± 0.60	1.54 ± 1.31

21.	Adol Drops (99)	18	5.8	96.9 ± 0.30	1.05 ± 1.02
22.	Adol Drops (104)	14	5.7	95.5 ± 0.25	0.85 ± 0.85
23.	Adol Suppositories (037)	33	-	86.5 ± 1.21	4.1 ± 0.65
24.	Fevadol Tablet (18635)	16	-	100.6 ± 0.80	Nil

Application of Developed Method in the Post-Marketing Stability Testing

The proposed stability-indicating HPLC method was applied for the determination of (I) and its degradation product (II) in syrups, drops, suspensions, suppositories and tablets. The results are summarized in Table 3. It is worth mentioning that all collected samples except tablets, were found to contain (II).

It was interesting to find that in aged syrups, the level of degradation product was high. The range varied from 0.7 - 3.25% in the case of 'Adol' Syrup and drops which were stored under room temperature for a period ranging from 14 - 26 months. All of these samples failed according to limit given by BP (NMT 0.5%). However, the acetaminophen content ranged between acceptable limits 95.1 - 98.1% (BP Limit: labelled amount 95 - 105%). Hence, it was concluded that although all the samples passed different quality control tests such as pH, consistency, and appearance, on the basis of 4-aminophenol (II) levels, these samples apparently expired before their labelled expiration date of 3 years.

The results of different tests and assay of 'Fevadol' syrups (age range 9-30 months) revealed that all of the batches tested complied with the specifications. However, the levels of the hydrolytic product assessed by the proposed method confirmed that the hydrolytic product (II) increased with the age of the sample. In 5 batches with age above 12 months, the level of (II) was found to range between 0.65 - 2.8% which is above the limit specified by BP.

In case of "Adol" suppositories aged 33 months it was observed that concentrations of both (I) and (II) dropped beyond the limits given by BP. However, "Fevadol" tablets aged 16 months were found to contain no degradation product (II).

CONCLUSIONS

The results of the present study revealed that the developed HPLC method is suitable for stability monitoring of acetaminophen containing preparations. This method has the advantage of determining (I) and its hydrolytic product (II) in a single run with the capability of quantifying very low concentrations of 4-Aminophenol (0.1 µg/ml). Furthermore, it is recommended that a limit for the degradation product be included in the specification of all acetaminophen preparations.

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