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A quantitative and qualitative high performance liquid chromatographic determination of acetaminophen and five of its *para*-substituted derivatives

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Abstract

An accurate HPLC determination of acetaminophen in tablet dosage form is reported, together with an effective separation of five of its *para*-substituted derivatives using an isocratic reversed-phase system. The column consisted of an octadecylsilane 10 μm stationary phase. The mobile phase was a mixture of acetonitrile and water (7:3). The retention times for acetaminophen, *O*-acetylacetaminophen, *O*-ethylacetaminophen, *O*-(2-nitrobenzenesulphonyl)acetaminophen, *O*-benzylacetaminophen and *O*-(3,5-dinitrobenzoyl)acetaminophen were 2.83, 3.25, 3.56, 3.78, 4.37 and 6.03 min, respectively.

Keywords: Acetaminophen; *para*-substituted derivatives; Isocratic reversed-phase system

1. Introduction

Several methods describing the determination of acetaminophen by HPLC have been reported. As early as 1970, Henry [1] described a rapid high-pressure anion exchange chromatographic procedure for the determination of acetaminophen in analgesic tablets. The following year, Stevenson [2] reported on an improved procedure for determining acetaminophen in a wide range of analgesic tablets. The majority of reports describing the determination of acetaminophen used C18 reversed-phase columns [3–6] but normal-phase silica columns [7] together with cyanopropylsilane columns [8–11] which, incidentally, can be used in normal-phase and reversed-phase chromatography, have been reported [12]. A number of HPLC methods describing the determination of acetaminophen used buffered mobile phases [8–10], while others used mobile phases consisting of ion-pairs [13,14]. Mobile phases consisting of buffers or ion-pair mixtures are found to be essential for the effective separation of acetaminophen from other actives and possible excipient interference in various dosage forms. Unbuffered mobile phases can sometimes cause fluctuation in the retention times of the acetaminophen which may be attributed to high concentrations of certain

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Table 1
Repeatability of the Test Procedure for Acetaminophen at a concentration of 3 mg/100 ml[a]

Experiment	Sample	Response (Peak area)	Amount found (mg/100 ml)	Recovery (%)[b]	RSD
	1	37.54	3.03	101.00	
	2	37.43	3.02	100.70	
1	3	37.46	3.02	100.70	0.52
	4	37.48	3.03	100.90	
	5	37.20	3.00	100.00	
	6	37.13	2.99	99.70	
	1	37.08	2.97	99.00	
	2	36.98	2.96	98.70	
2	3	36.88	2.95	98.30	0.35
	4	37.00	2.96	98.70	
	5	37.20	2.98	99.30	
	6	37.12	2.97	99.00	

[a] The table shows the percent recovery of acetaminophen. System suitability was checked by injecting the standard six times in order to obtain consistent results.
[b] Average of two injections.

Table 2
Reproducibility of the test procedure for acetaminophen at a concentration of 3 mg/100 ml[a]

Experiment	Sample	Response (peak area)	Amount found (mg/100 ml)	Recovery (%)[b]	RSD
	1	37.55	3.08	102.60	
	2	37.68	3.09	103.00	
1	3	36.10	2.96	98.70	1.59
	4	37.74	3.09	103.00	
	5	37.34	3.06	102.00	
	6	37.20	3.05	101.70	
2	1	37.06	2.96	98.70	
	2	37.35	2.99	99.70	
	3	38.62	3.09	103.00	1.62
	4	37.30	2.98	99.30	
	5	37.90	3.03	101.06	
	6	38.07	3.05	101.70	

[a] The table shows the percent recovery of acetaminophen. System suitability was checked by injecting the standard six times in order to obtain consistent results.
[b] Average of two injections.

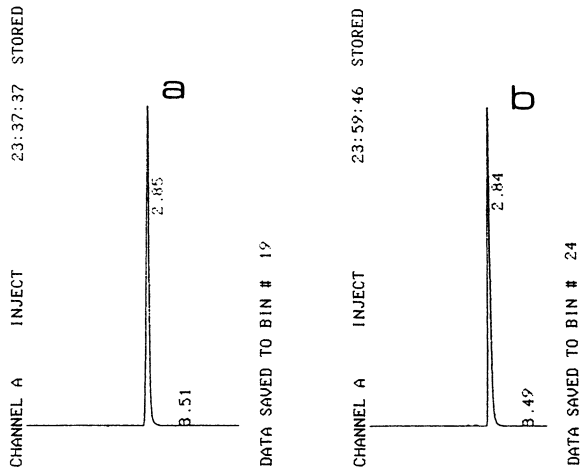


Fig. 1. (a) Chromatogram of Acetaminophen standard using a final concentration of 3 mg/100 ml and wavelength of detection of 254 nm. (b) Chromatogram of Acetaminophen sample using a final concentration of 3 mg/100 ml and wavelength of detection of 254 nm.

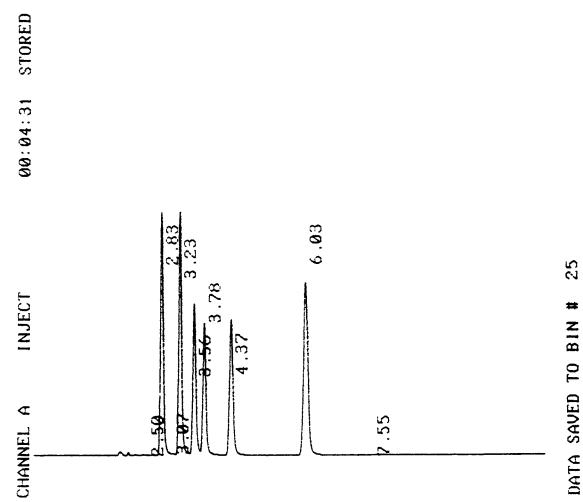


Fig. 2. Chromatogram of Acetaminophen, *O*-acetylacetaminophen, *O*-ethyl-acetaminophen, *O*-(2-nitrobenzenesulphonyl)acetaminophen, *O*-benzyl-acetaminophen and *O*-(3,5-dinitrobenzoyl)acetaminophen.

excipients. The use of buffers at concentrations lower than 0.1 M are usually required when acidic or basic compounds are analysed. Even though acetaminophen is a weak organic acid with a p*K*a of 9.5, a number of investigators have reported the successful separation of acetaminophen without the use of buffers [6,15–19].

In this paper, we report on a fast, effective and inexpensive method for assaying acetaminophen in tablets containing upto 500 mg of acetaminophen and approximately 100 mg of excipient, using an unbuffered isocratic acetonitrile/water (7:3) mobile phase at pH 6.3 and a Waters Symmetry C18 column. In addition to assaying acetaminophen, the method can be used to separate five *para*-substituted acetaminophen derivatives, together with acetaminophen in a single run.

2. Materials and methods

2.1. Reagents

USP grade acetaminophen was purchased from Sigma (USA); 3,5-dinitro-benzoylchloride and benzoylchloride from Fluka Chemika (Romania); 2-nitro-benzenesulphonylchloride and ethyl iodide from Acros Organics (USA); acetylchloride from Pancreac (Spain); absolute ethanol from Gainland Chemical Co. (UK); sodium metal from Avondale Laboratories (UK); nitromethane from Aldrich (USA); 3-bromopropene from Riedel-Dettaen (Germany); HPLC grade acetonitrile and water from Lab Scan Analytical Sciences (Ireland); magnesium stearate, maize starch, lactose, microcrystalline cellulose and methylcellulose were obtained from Sigma (USA). The five brands of acetaminophen tablets were obtained from The Jordanian Pharmaceutical Manufacturing Co. (Jordan); Hikma Pharmaceuticals (Jordan); The Arab Pharmaceutical Manufacturing Co. (Jordan); Smithkline Beecham (Ireland) and Brunel Trading Co. (UK).

2.2. Preparation of stock solution

A standard stock solution was prepared by dissolving acetaminophen (50 mg) in a 7:3 mixture of acetonitrile/water (solvent A) and making up to the mark in a 1000 ml volumetric flask with solvent A.

2.3. Preparation of stock solution (spiked with placebo)

A standard stock solution prepared by transferring acetaminophen (50 mg) to a 1000 ml volumetric flask, dissolved with solvent A was treated with placebo. The mixture was sonicated and then made up to the mark with solvent A.

2.4. Sample preparation (dosage form of the five brands of tablet)

The powered tablet equivalent to 50 mg of the active was transferred to a 100 ml volumetric flask and treated with 50 ml of solvent A. The mixture was sonicated for 10 min and then made up to the mark with solvent A. After further mixing, 10 ml

Table 3
The comparison between the retention time of acetaminophen and five related derivatives

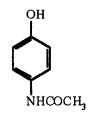
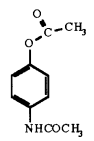
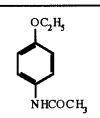
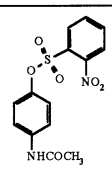
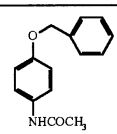
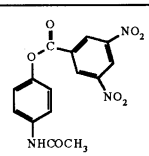
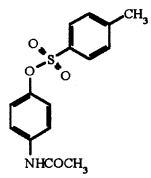
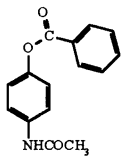
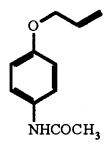
Structure of compound	Retention time (min)	Peak label
	2.83	1
	3.23	2
	3.56	3
	3.78	4
	4.37	5
	6.03	6

Table 4
The three additional derivatives of Acetaminophen that were examined using the analytical procedure.*

Structure	Name of derivative
	O-(4-methylbenzenesulphonyl)acetaminophen
	O-benzoylacetaminophen
	O-(prop-3-enyl)acetaminophen

* Both *O*-(4-methylbenzenesulphonyl)acetaminophen and *O*-benzoylacetaminophen co-eluted with *O*-benzylacetaminophen. The *O*-(prop-3-enyl)acetaminophen co-eluted with *O*-(2 nitrobenzenesulphonyl)acetaminophen.

of the mixture was centrifuged at 4000 rpm for 15 min and 3 ml of the supernatant was transferred to a 50 ml volumetric flask and made up to the mark with solvent A.

2.5. Sample preparation (used to test for linearity)

2.5.1. Standard solution

Five concentrations were prepared from the stock solution ranging from 1–5 mg/100 ml.

2.5.2. Sample solutions

Five concentrations ranging from 1–5 mg/100 ml were prepared from the stock solution spiked with placebo, after the removal of undissolved excipients by centrifugation.

2.5.3. Mobile phase preparation

Acetonitrile-water (70:30, v/v) (pH* 6.3). The mobile phase was prepared by transferring 300 ml of water to a 1000 ml volumetric flask and making up to the mark with acetonitrile. The mixture was filtered under vacuum and further degassed by sonication.

2.6. Preparation of acetaminophen derivatives

Sodium metal (0.46 g, 19.8 mM) was added to absolute ethanol (15 ml) in a 2-necked round bottom flask equipped with a reflux condenser. Once all the sodium had reacted to produce sodium ethoxide, the solution was stirred for a further 10 min after which time acetaminophen powder was added. The acetaminophen solubilised almost instantly and the reaction mixture was stirred at room temperature for 30 min. The corresponding alkylating or acylating reagent (19.8 mm) was dissolved in absolute ethanol (10 ml) and added dropwise over a 10 min period. The reaction mixture was stirred for a further 15

min and then gently refluxed, monitored by TLC. After the reaction was complete, it was cooled and treated with water (50 ml). The precipitated product was filtered and washed with water (3 × 40 ml) and then recrystallised from absolute ethanol. The solid was dried and stored in a vacuum desicator. All compounds were confirmed by ^{1}H-nmr, ir and m.pt.

The preparation of *O*-acetylacetaminophen required a modified procedure due to the highly reactive nature of acetylchloride. The procedure involved dissolving acetaminophen (3 g, 19.8 mM) in nitromethane (20 ml) and then adding a slight excess of acetylchloride (22 mM) at room temperature. The reaction mixture was stirred at room temperature, monitored by TLC and after 1 h the reaction was complete. The nitromethane and traces of acetylchloride were removed by rotary evaporation and the product was recrystallised from absolute ethanol.

2.6.1. Equipment

The HPLC system used in the study consisted of a GBC LC 1110 programmable pump (Australia), a GBC LC 1210 uv/vis programmable detector (USA) and a Data Jet Integrator/printer (USA).

2.6.2. Chromatograhic conditions

The chromatographic column used was a Waters Symmetry C18, 250 × 4.6 mm I.D., particle size 10 μm connected to a Waters C18 Symmetry guard column. The size of the loop was 20 μl. The mobile phase consisted of seven parts by volume of acetonitrile and three parts by volume of water. Detection was measured at 254 nm and the pump flow rate was 1 ml/min. The chromatographic analyses were carried out at ambient temperature.

3. Results and discussion

In order to assay acetaminophen accurately, five concentrations of both the sample and standard solutions ranging from 33–167% of the theoretical assay concentration were analysed. System suitability was performed before conducting the determinations of the five concentrations. This was achieved after obtaining consistent results from six injections of the standard covering all five concentrations. The average percent recovery of acetaminophen for the five sample concentrations was 100.9% with a relative standard deviation of 0.99%. The correlation coefficient was 0.9992 with a slope of 15.49 and an intercept of −0.384. The RDS of the response factor for the five standards was 1.27. Generally, linearity [20] is confirmed if the RSD of the response factor is less than 2.0%.

Repeatability was determined by preparing two sets of six synthetic mixtures together with freshly prepared standards having the final concentration of 3 mg/100 ml. The average percent recovery for the active was 100.5 and 98.83% with RSD's of 0.52 and 0.35%, respectively. The results are shown in Table 1.

Reproducibility was achieved by preparing two sets of synthetic mixtures similar to that carried out for repeatability but the determinations were performed on consecutive days. The average percent recovery for the active was 101.8 and 100.6% with RSD's of 1.59 and 1.62%, respectively. The results are shown in Table 2.

Fig. 1a and b are the chromatograms of acetaminophen standard and sample, respectively. The active elutes at retention time 2.85. The excipients, which are a combination of magnesium stearate, maize starch, lactose, microcrystalline cellulose and methyl cellulose, do not interfere with the determination of the acetaminophen. All five brands of the acetaminophen tablet weighing on average, 600 mg and consisting of 500 mg of active were analysed successfully using the analytical procedure and produced consistent results to that of the synthetic sample. In addition to the method being used to accurately measure the assay of acetaminophen, five *para*-substituted derivatives of acetaminophen together with acetaminophen were separated simultaneously in a single run. The derivatives were synthesised by a standard one-step substitution of acetaminophen, as described by Vogel [21]. The general experimental procedure is described in the experimental section. Each derivative was quantitated both, separately, and as a mixture ranging from 33–167% of the theoretical assay concentration of

acetaminophen. Fig. 2 shows the chromatogram of acetaminophen, *O*-acetylacetaminophen, *O*-ethylacetaminophen, *O*-(2-nitrobenzenesulphonyl)-acetaminophen, *O*-benzylacetaminophen and *O*-(3,5-dinitrobenzoyl)acetaminophen eluting at retention times of 2.83, 3.25, 3.56, 3.78, 4.37 and 6.03 min, respectively. The results are shown in Table 3. Attempts to resolve an additional three derivatives of acetaminophen in the same run, proved difficult because of either co-elution or overlap of peaks. For example, the *O*-(4-methylbenzenesulphonyl)acetaminophen and *O*-benzoylacetaminophen coeluted with *O*-benzylacetaminophen, while *O*-(prop-3-enyl)acetaminophen coeluted with *O*-(2-nitrobenzenesulphonyl)acetaminophen. The three derivatives are shown in Table 4.

4. Conclusion

The developed method can be used to quantify acetaminophen in tablet dosage forms containing as much as 500 mg of the active with accuracy and precision. The method is linear, reproducible, sensitive, selective, rugged, economical, easy to perform and can be used to separate a number of *para*-substituted derivatives of acetaminophen. Further work is being carried out to determine whether the method is stability indicating with respect to acetaminophen.

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