

RAPID ANALYSIS OF CAFFEINE AND PARACETAMOL USING MICRO-LIQUID CHROMATOGRAPHY WITH MIXED MODE MONOLITHIC COLUMN

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Abstract

This work developed a simple and rapid micro-liquid chromatographic method using a synthesized monolithic capillary column for analysis of caffeine and paracetamol in pharmaceutical drugs. The monolithic column was synthesized from methacrylic acid and ethylene dimethacrylate monomers. Due to the anionic and hydrophobic properties of the polymeric stationary phase, complete separation of the two compounds was achieved by addition of triethylamine into the acetonitrile-aqueous buffer (pH 10.0) mobile phase using a micro-liquid chromatograph with uv-visible detector. Under the optimal condition, the two compounds were separated in less than 3 min. Good linearity in the concentration range of 1-50 mg/L ($r^2 > 0.997$) with detection limits of 0.05 mg/L for paracetamol and 0.30 mg/L for caffeine were obtained. The method was successfully applied to the determination of caffeine and paracetamol in drug samples with percent recoveries of 102 and 99, respectively.

Keywords: Caffeine, paracetamol, monolithic column, micro-liquid chromatography, triethylamine additive

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Introduction

Paracetamol (N-acetyl-p-aminophenol, PARA) is probably the most widely used drug to reduce fever, pain and headache. The compound has been found to be safe with no serious side effect on human health. However, overdose and long term use of this drug could lead to kidney and liver damage (Mahadevan *et al.*, 2006; Pakravan *et al.*, 2007).

Caffeine (1,3,7-trimethylxanthine, CAF) is a central nervous system stimulant widely used as a flavoring agent in foods and beverages, such as energy drinks, cola and soft drinks. In addition, CAF is also used as an adjuvant analgesic drug; a combination of CAF and PARA is commonly used for treatment of migraine headache, muscle ache and toothache (Diener *et al.*, 2011; Straube *et al.*, 2011). It is believed that the absorption of PARA can be accelerated by combining with CAF and the pain tolerance is increased (Cunha *et al.*, 2015).

In order to simultaneously analyze CAF and PARA, several techniques have been reported including capillary electrophoresis (Cunha *et al.*, 2015), spectrophotometric methods (Dinc *et al.*, 2001; Alves and Poppi, 2009), high performance liquid chromatography (Kartal, 2001; Wand *et al.*, 2007; Kullilow and Verushkin, 2008; Acheampong *et al.*, 2016;), chemometric methods (Khoshayand *et al.*, 2008; Topkafa *et al.*, 2016) and electrochemical methods (Chaves *et al.*, 2015; Tefera *et al.*, 2016; Machado *et al.*, 2017; Minh *et al.*, 2017; Silva *et al.*, 2017). High performance liquid chromatography is the most popular technique used for this application due to the high separation efficiency, good reproducibility and ease of operation. A monolithic column providing unique properties of high porosity, wide operating pH range, ease of preparation and modification has been coupled with HPLC for various applications (Svec and Huber, 2006; Samanidou and Karageorgou, 2011; Zhu and Row, 2012; Pfaunmille *et al.*, 2013) including PARA and CAF (Pistos and Stewart, 2004; Koblova *et al.*, 2012). However, the applications were based on commercial silica-based monolithic columns with regular HPLC

system which required large solvent consumption.

In this work, we aim to show the potential of coupling a lab-made monolithic column with micro-LC with UV detection as a simple and green analytical method for simultaneous analysis of PARA and CAF in drug sample. Selectivity of the synthesized mixed mode monolithic column for PARA and CAF and effect of triethylamine were investigated and demonstrated.

Materials and Methods

Chemicals and Reagents

HPLC grade methanol (MeOH) and acetonitrile (ACN) were obtained from QRêc (New Zealand). Phosphoric acid (H_3PO_4), potassium dihydrogen phosphate (KH_2PO_4), di-sodium hydrogenphosphate (Na_2HPO_4) and sodium hydroxide were from Carlo Erba (Rodano, MI, Italy). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), azobisisobutyronitrile (AIBN), and 3-(trimethoxysilyl)propyl methacrylate (γ -MAPS) were obtained from Aldrich (Steinheim, Germany). Glacial acetic acid (CH_3COOH) was supplied from Labscan (Bangkok, Thailand). Fused silica capillary (100 μm i.d and 360 μm o.d.) was supplied by Agilent (Santa Clara, US). Caffeine (CAF) and paracetamol (PARA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Stock and working solutions of caffeine and paracetamol were prepared daily in RO water.

Phosphate buffer concentration of 20 mM, were prepared by dissolving 123 μL of H_3PO_4 , 0.2722 g KH_2PO_4 and 0.2839 g Na_2HPO_4 in ca. 90 mL RO water and the pH adjusted to 2.0, 7.0, or 10.0, respectively, with 0.1 M NaOH before making up to volume in 100 mL volumetric flasks. For the 20 mM acetate buffer pH 4.0, 116 μL glacial acetic acid was diluted with ca. 90 mL RO water, its pH adjusted with 0.1 M NaOH and then made up to volume in a 100 mL volumetric flask. The buffer solutions were filtered through 0.45 μm cellulose membrane before use.

Instrumentation

The microliquid chromatograph consisted of a LC pump Model 582 (Shimadzu, Japan) equipped with a six port micro injector with 250 nL sample loop Model C2N-4346 (Valco, USA) and a UV-visible detector Model 785A (Perkin Elmer, USA) with data processing system from eDAQ (Australia). The flow rate of the mobile phase was 2 $\mu\text{L}/\text{min}$ and the detection wavelength was set at 272 nm. Hot air oven Model INB-400 (Mettler, Germany) was used for thermal polymerization of the monolith.

Preparation of Monolithic Capillary Column

The monolithic capillary column was prepared as reported previously (Chaloemsuwattanakan *et al.*, 2016). The inner wall of a 38 cm fused silica capillary was treated with 50% (v/v) γ -MAPS in MeOH before filling in with a solution comprised of 29.5% (w/w) MAA, 3.4% (w/w) EDMA, 8.1% (w/w) toluene, 59.0% (w/w) 1-decanol and 1% (w/w) AIBN (%by weight compared with total weight of monomers, MAA and EDMA). The two ends of the capillary were then sealed with rubber septa and the capillary placed in a hot air oven at 60°C for 4 h. After the polymerization step, the synthesized monolithic capillary column was washed with methanol at a flow rate of 2 $\mu\text{L}/\text{min}$ for 1 h to remove the unreacted compounds and solvents. A UV-detection window was created at the outlet end of the column by burning off the imide coating of the capillary with a burner to obtain an effective length of 32 cm before installing the capillary column on the micro-LC system.

Sample Preparation

Pharmaceutical drugs (Norshin white tablets) were purchased from a local shop in Japan. Four tablets of the sample were weighed before grinding to obtain homogeneous samples. Triplicate sets of 0.0100 g sample were dissolved in ca. 4 mL RO water. The suspended samples were sonicated at room temperature for 10 min and made up to 5.00 mL volume with RO water. The sample solutions were filtered through syringe filter

(pore size 0.2 μm) before injecting into the micro-LC system.

Results and Discussion

Effect of Triethylamine on The Separation of PARA and CAF on The MAA-EDMA Monolithic Capillary Column

The monolith containing polar carboxylic groups on the surface with relatively nonpolar ester hydrocarbon structure, was chosen for this work as it has been shown that this polymeric stationary phase provided high separation efficiency for polar compound (Chaloemsuwattanakan *et al.*, 2016). The interaction between the analyte and stationary phase can therefore be hydrophobic, hydrophilic or coulombic. The two common organic solvents used in LC, MeOH and ACN, were first investigated for the separation of PARA and CAF. At the same ratio of organic to water (20:80, v/v) methanol produced better selectivity than acetonitrile (Figure 1(a)). This is not unexpected since acetonitrile is a stronger mobile phase (for reversed-phase mode) leading to shorter retention and co-elution of the two compounds (Figure 1(b)). However, the acetonitrile/water mobile phase

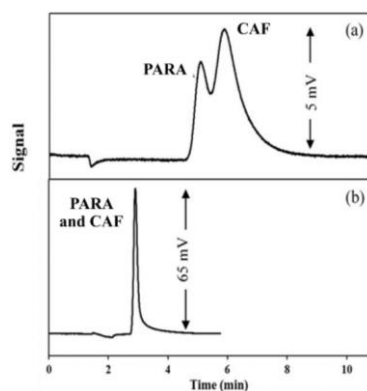


Figure 1. Separation of PARA and CAF mixture on MAA-EDMA monolithic column, mobile phase (a) 20:80 (v/v) MeOH:water and (b) 20:80 (v/v) ACN:water, injection volume of 250 nL, UV detection at 272 nm, flow rate of 2 $\mu\text{L}/\text{min}$

was chosen for further investigation due to the sharper peak and higher signal.

The effect of pH on the separation was carried out at pH 2.0, 4.0, 7.0, and 10.0 (data not shown) using ACN:20 mM buffer at ratio of 20:80 (v/v). Increasing in selectivity was found at high pH. At pH > 7.0 fraction of the surface of the stationary phase is negatively charged due to ionization of the carboxylic groups. Nevertheless, caffeine eluted slightly earlier than PARA even at pH 10.0 where PARA is ca. 75% dissociated (pKa = 9.5).

In order to improve the selectivity at pH 10.0, triethylamine (TEA) was added to the mobile phase. TEA has been employed in reversed phase chromatography as an end-

capping agent or as an ion-pairing reagent for anionic analyte (Prasad and Ragan, 1994; Gong, 2015). At this pH, TEA (pKa = 10.75) mostly exists as its protonated cation whereas PARA (pKa = 9.5) and the methacrylate groups on the monolithic surface are dissociated to its anionic form. The ion-pair formation leads to complete separation of PARA and CAF as shown in Figure 2(a). The elution order is as found for RP column. At pH 7.0 PARA is now neutral and ion-pairing cannot occur; there is no selectivity between PARA and CAF (Figure 2(b)).

The concentration of TEA was found to be an important parameter. Separation selectivity increased with increasing TEA concentration until the concentration of TEA reached 1 mM (data not shown). Good system stability and short equilibrating time was achieved up to 2 mM; at higher concentration solubility of the TEA in the mobile phase became a problem. The mobile phase of 20:80 (v/v) ACN:20 mM phosphate buffer pH 10.0, containing 2 mM TEA, was selected.

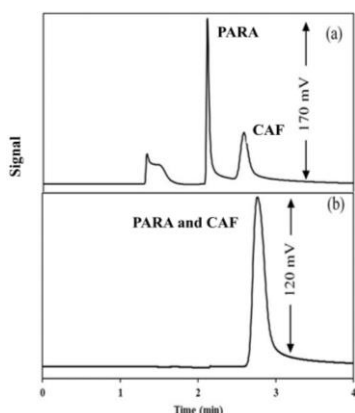


Figure 2. Separation of PARA and CAF mixture on MAA-EDMA monolithic column, mobile phase of 20:80 (v/v) ACN:20 mM phosphate buffer containing 2 mM triethylamine. (a) pH 10.0 and (b) at pH 7.0. Other conditions are as described in Figure 1

Analytical Features of The Developed Micro-LC Method

Linear calibration curves were constructed in the range of 1-50 mg/L for both compounds. The LOD and LOQ were defined as the concentrations providing signal-to-noise (S/N) ratio of 3 and 10, respectively. Intra-day precision was determined by injecting standard solutions containing 10 mg/L of PARA and CAF for 7 replicate injections on the same day and for three days for inter-day precision. The results of the analytical features study are given in Table 1. Compared with other

Table 1. Analytical features of the developed micro-LC method

	PARA		CAF	
	t_r	Peak area	t_r	Peak area
Intraday precision (%RSD, n=7)	0.82±0.02	1.70±0.70	0.78±0.02	1.61±0.65
Interday precision (%RSD, n=3)	1.95±0.05	3.43±1.41	3.82±0.11	3.65±1.50
Concentration range (mg/L)	1 - 50		1 - 50	
Calibration equation Slope	$y = (5.3 \pm 0.1)x + (4.7 \pm 3.5)$		$y = (7.9 \pm 0.1)x + (2.8 \pm 2.0)$	
r^2	0.9978		0.9997	
LOD (mg/L)	0.05		0.3	
LOQ (mg/L)	0.17		1	

Table 2. Analytical results and recovery of PARA and CAF in pharmaceutical drug

Sample	Concentration of PARA (mg/tablet)				Concentration of CAF (mg/tablet)			
	Label value	Added	Found value	Recovery (%)	Label value	Added	Found value	Recovery (%)
Combined drug	160.00	0	134.49±1.17	-	70.00	0	34.65±0.08	-
		99	235.04±1.73	102		99	132.95±0.19	99

methods, our method was fast (separation time of 3 min) and simple, with throughput up to 20 samples/h and low solvent consumption of 120 μ L for 20 samples.

Application

The method was applied to the determination of caffeine and paracetamol in Norshin white tablets sample containing PARA and CAF (Figure 3). The analysis results and %recovery are shown in Table 2. It is noted that the experimental measured values differ with the label values. However, the accuracy of the method in terms of %recovery was in the acceptable range of 102 and 99 for CAF and PARA, respectively. Moreover, high throughput analysis could be achieved of 20 samples/h.

Conclusions

Paracetamol and caffeine were separated in 3 min using the synthesized MAA-EDMA monolithic capillary column with mobile phase of 20:80 v/v ACN:20 mM phosphate buffer pH 10.0 containing 2 mM triethylamine. The mobile phase pH and TEA additive were crucial parameters for successful separation. The method was applied to the determination of paracetamol and caffeine in pharmaceutical drug. Our micro-separation method was fast, cost effective, stable and environmentally friendly.

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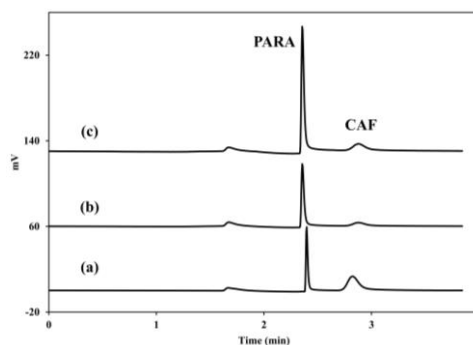


Figure 3. Chromatogram for (a) mixed standard 10 mg/L PARA and 10 mg/L CAF (b) pharmaceutical drug tablet containing PARA and CAF and (c) pharmaceutical drug tablet containing PARA and CAF spiked 99 mg/tablet for PARA and 99 mg/tablet for CAF. Other conditions are as described in Figure 2(a)

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