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Development of Extraction and Detection Method for a Chemotherapeutic Drug with Phenytoin in Biological Samples

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Abstract

Dexamethasone is classified as a corticosteroid and is commonly used among cancer patients to decrease the amount of swelling around the tumor. Among patients with cancer, in particular brain tumors, seizures can become a daily routine in their everyday lives. To counteract the seizures, an antiepileptic drug such as phenytoin is administered to act as an anticonvulsant. Phenytoin and dexamethasone are frequently administrated concurrently to brain cancer patients. A previous study has shown that phenytoin serum concentration decreases when administrated concurrently with dexamethasone. Thus, it is important to monitor the concentration of these two drugs in biological samples to ensure that the proper dosages are administrated to the patients. This study aims to develop an effective extraction and detection method for dexamethasone and phenytoin. A reverse-phase high-performance liquid chromatography (HPLC) method with UV/Vis detection has been developed to separate phenytoin and dexamethasone at 219 nm and 241 nm respectively from urine samples. The mobile phase consists of a mixture of 0.01 M KH_2PO_4, acetonitrile, and methanol adjusted to pH 5.6 (48:32:20) and is pumped at a flow rate of 1.0 mL/min. Calibration curves were prepared for phenytoin and dexamethasone ($r^2 > 0.99$). An efficient solid-phase extraction (SPE) method for the extraction of dexamethasone and phenytoin from urine samples was developed with the use of C-18 cartridges. The percent recovery for phenytoin and dexamethasone is 95.4% (RSD = 1.15%) and 81.1% (RSD = 3.56%) respectively.

Keywords

Dexamethasone, Phenytoin, Cancer, Drug Interaction, Solid Phase Extraction

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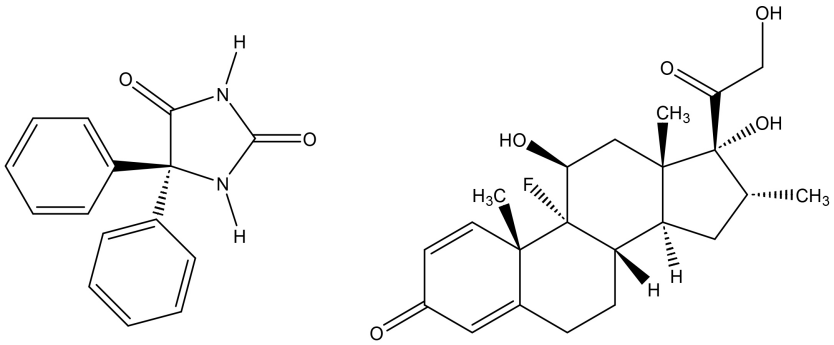
1. Introduction

Dexamethasone is a corticosteroid commonly used in anti-cancer therapy. For patients who have brain tumors, dexamethasone is used to reduce inflammation caused by the tumor [1] [2]. Studies have shown that many brain cancer patients suffer from epileptic seizures [3]-[6]. Thus, dexamethasone is frequently administrated along with seizure treatment drugs such as phenytoin [7] [8]. Phenytoin has a narrow therapeutic window [9]. For example, it has been shown that increased brain P-glycoprotein (P-gp) may contribute to resistance to phenytoin [10]. Previous study shows that phenytoin decreases the levels of dexamethasone in patients [11] [12]. On the other hand, the effect of dexamethasone on phenytoin is inconclusive. Both decreases [13]-[16] and increases [17] in the levels of phenytoin have been observed when dexamethasone is administrated concurring with phenytoin. Due to the various interactions among these two drugs, it is important to monitor the concentration of them to determine the ideal dosages among cancer patients. For example, Skrabalak and Maylin detected the concentration of dexamethasone and its major metabolite in horse urine by using radiolabeled dexamethasone (9-fluoro-16α-methyl-11β, 17, 21-trihydroxy-1,4-pregnadiene-3, 20-dione) and thin-layer chromatography [18]. In addition, Yaripour *et al.* detected phenytoin in human serum and urine by using electromembrane extraction (EME) followed by High Performance Liquid Chromatography with UV detection [19]. This study aims to develop an effective extraction and detection method for dexamethasone and phenytoin simultaneously (see **Figure 1** for their structures) in urine samples.



Phenytoin Dexamethasone

Figure 1. Structure of dexamethasone and phenytoin.

2. Experimental

2.1. Chemicals and Instrumentation

Dexamethasone and phenytoin were purchased from Sigma Aldrich Inc (St. Louis, MO, U.S.A.). A Shimadzu HPLC system is used (Kyoto, Japan). It consists of a photodiode array (Kyoto, Japan, SPD-M 10A VP), two pumps (LC-10AT), and a controller (SCL-10A VP). It is coupled to a C18 column (Phenomenex, 250 × 4.6 mm, 5-micron particles) through a high-pressure injection valve from Rheodyne Inc. (Cotati, CA, USA, model RH-7725i) with a 10 μL sample loop. The detection

wavelength for phenytoin and dexamethasone is 219 nm and 241 nm respectively.

2.2. HPLC Condition

The mobile phase consists of a mixture of 0.001 M KH_2PO_4, acetonitrile, and methanol adjusted to pH 5.6 (48:32:20) and is pumped at a flow rate of 1.0 mL/min. Retention time for dexamethasone and phenytoin was determined by injecting each compound individually to the HPLC system. The identification of dexamethasone and phenytoin from the extracted samples was determined by the corresponding retention time.

2.3. Selection of Detection Wavelength

Initially, the detection wavelength for both dexamethasone and phenytoin was set at 230 nm. After further study, it was determined that the optimum detection wavelength for phenytoin and dexamethasone is 219 nm and 241 nm respectively.

2.4. Calibration

A phenytoin stock solution (concentration = 504 μg/mL) and a dexamethasone stock solution (concentration = 598 μg/mL) were prepared by accurately weighing out the corresponding substance and using methanol as the solvent. Five standard solutions were prepared from these two stock solutions with a concentration range from 25.2 - 1.57 μg/mL for phenytoin and 29.9 - 1.87 μg/mL for dexamethasone. Each standard solution was injected three times.

2.5. Preparation of Samples

20 mL of urine sample was spiked with 1 mL of the phenytoin stock solution (concentration = 563 μg/mL) and 1 mL of the dexamethasone stock solution (concentration = 578 μg/mL). The resulting phenytoin and dexamethasone concentrations in the spiked sample are 28.15 μg/mL and 28.90 μg/mL respectively. The spiked sample was vortexed for 7 min. A blank was prepared similarly with 20 mL of distilled water.

2.6. Solid Phase Extraction

Extractions were performed with the 3 mL Strata C-18-E cartridges (Phenomenex) and the 24-port, vacuum manifold (Phenomenex).

The cartridge is conditioned with: 1) 3.0 mL of methanol (MeOH), 2) 2.0 mL of DI water, and 3) 2.0 mL of 0.1 M phosphate buffer at pH 6. The extraction procedure is as follows:

1) 3 mL of the spiked urine sample is loaded onto the cartridge, 2) washed with 3.0 mL of distilled water, 3) washed with 2.0 mL of 20% MeOH (v/v), and 4) finally eluted with 3.0 mL of MeOH.

Eluted samples were dried under a steady stream of nitrogen gas. Each dried sample was re-dissolved in 1.0 mL of methanol. The dissolved sample was then filtered through a 0.45 μm syringe filter prior to injection into the HPLC system. Each dissolved sample was injected 3 times.

2.7. Comparison Study on Different Spiking Levels

20 mL of the urine sample was spiked with 1 mL of the phenytoin stock solution (concentration = 112.6 µg/mL) and 1 mL of the dexamethasone stock solution (concentration = 115.6 µg/mL). The resulting phenytoin and dexamethasone concentrations in the spiked sample were 5.630 µg/mL and 5.780 µg/mL respectively.

2.8. Percent Recovery Study

Percent recovery studies for the simultaneous extraction of dexamethasone and phenytoin were performed by spiking urine samples and blanks at two concentration levels as described above. Precision was evaluated by inter-day (3 days and intra-day (3 samples) studies.

3. Results and Discussion

3.1. Calibration

Calibration curves for phenytoin and dexamethasone are shown in Figure 2(a) and Figure 2(b). Both have a r^2 value greater than 0.99.

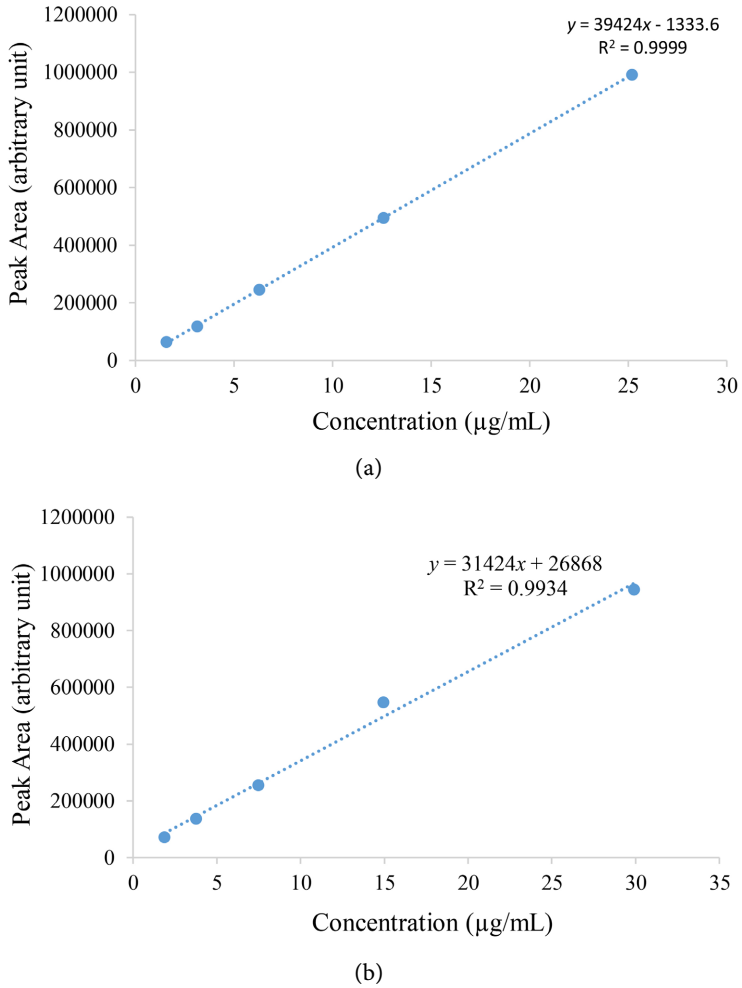
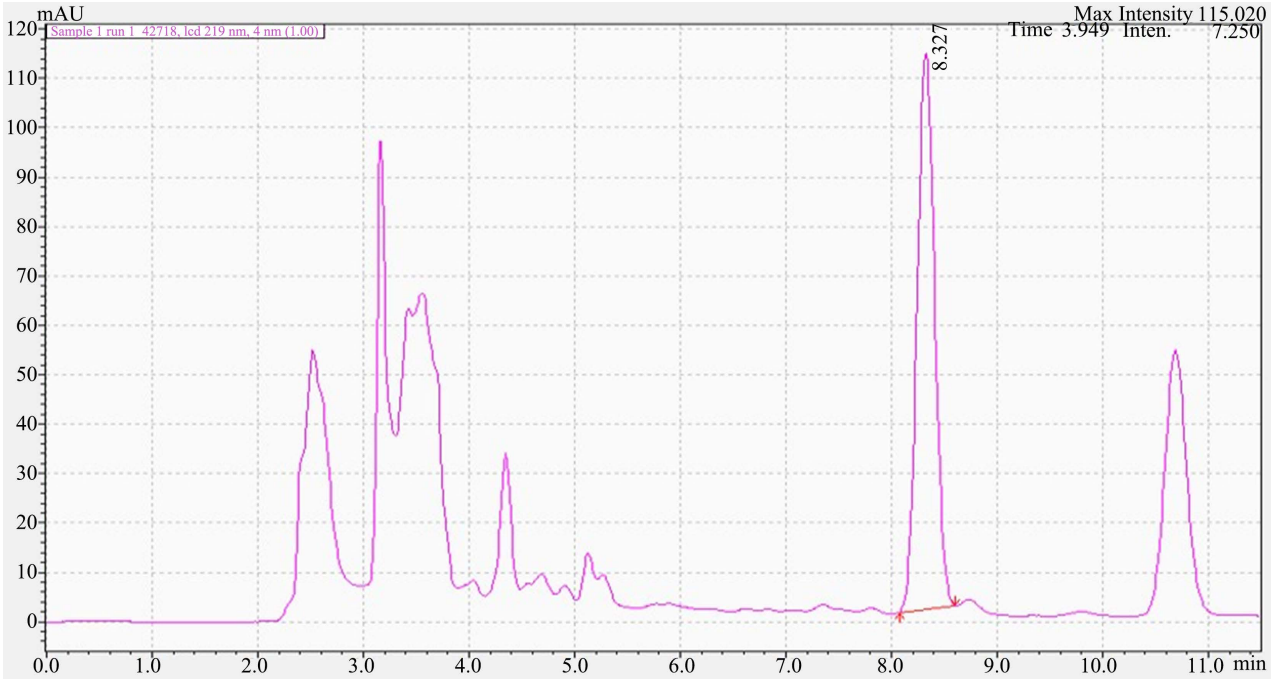


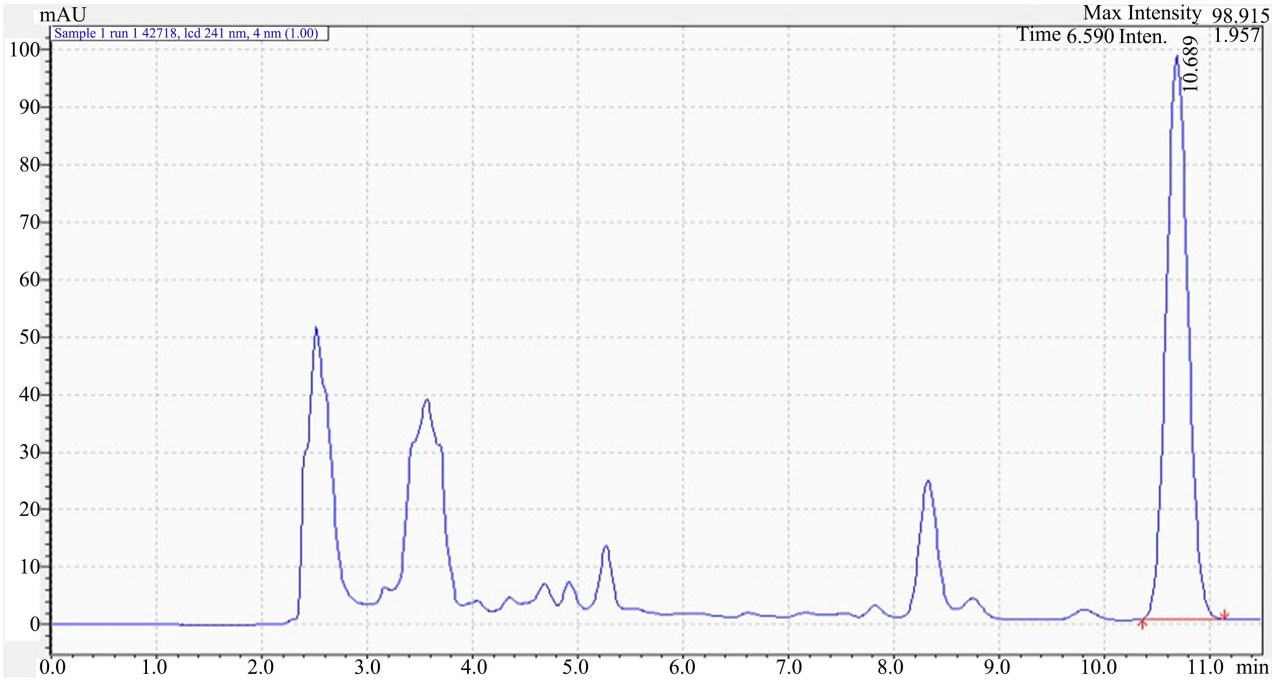
Figure 2. (a) Calibration curve for phenytoin; (b) Calibration curve for dexamethasone.

3.2. Solid Phase Extraction

Chromatograms of the extracted urine sample are shown in Figure 3(a) and Figure 3(b). Retention times for phenytoin (detected at 219 nm) and dexamethasone (detected at 241 nm) are 8.33 minutes and 10.69 minutes respectively.



(a)



(b)

Figure 3. (a) Chromatogram of extracted sample: Detection wavelength = 219 nm; (b) Chromatogram of extracted sample: Detection wavelength = 241 nm.

3.3. Percent Recovery and Reproducibility

Percent recovery was calculated as the ratio between the amount extracted from the spiked urine samples and the amount extracted from the blank. Results of the inter-day and intra-day studies at the two spiking levels are shown in Table 1 and Table 2. Inter-day percent recovery for phenytoin and dexamethasone is 95.4% and 81.1% respectively at the corresponding spiking level of 5.640 μg/mL and 5.780 μg/mL. A similar percent recovery for phenytoin and dexamethasone was observed at the higher spiking level. Both the inter-day and intra-day studies have a relative standard deviation (RSD) of less than 4%. This shows that the developed extraction method has good precision.

Table 1. Summary of percent recovery for inter-day and intra-day studies: spiked concentration = 28.15 μg/mL and 28.90 μg/mL respectively for phenytoin and dexamethasone.

	Percent Recovery				
Spiked Concentration	**Day 1** Intra-day average (n = 3) and Relative Standard Deviation (RSD)	**Day 2** Intra-day average (n = 3) and Relative Standard Deviation (RSD)	**Day 3** Intra-day average (n = 3) and Relative Standard Deviation (RSD)	**Inter-Day Average**	**Inter-day Relative Standard Deviation (RSD)**
Phenytoin 28.15 μg/mL	93.4% (RSD = 1.42%)	94.5% (RSD = 3.63%)	92.1% (RSD = 2.99%)	93.3%	2.68%
Dexamethasone 28.90 μg/mL	78.3% (RSD = 2.63%)	77.8% (RSD = 3.36%)	78.3% (RSD = 2.48%)	78.1%	2.82%

Table 2. Summary of percent recovery for inter-day and intra-day studies: spiked concentration = 5.630 μg/mL and 5.780 μg/mL respectively for phenytoin and dexamethasone.

	Percent Recovery				
Spiked Concentration	**Day 1** Intra-day average (n = 3) and Relative Standard Deviation (RSD)	**Day 2** Intra-day average (n = 3) and Relative Standard Deviation (RSD)	**Day 3** Intra-day average (n = 3) and Relative Standard Deviation (RSD)	**Inter-Day Average**	**Relative Standard Deviation (RSD)**
Phenytoin 5.630 μg/mL	96.7% (RSD = 1.59%)	95.5% (RSD = 1.27%)	94.0% (RSD = 0.583%)	95.4%	1.15%
Dexamethasone 5.780 μg/mL	82.8% (RSD = 1.96%)	78.3% (RSD =7.95%)	82.2% (RSD =0.769%)	81.1%	3.56%

4. Conclusions

An efficient and simple solid-phase extraction method was developed for simultaneous extraction of phenytoin and dexamethasone from urine example was developed. Percent recovery for phenytoin and dexamethasone was 95.4% and 81.1%. Similar percent recovery for both drugs was observed at a different spiking level. Results from the inter-day and intra-day studies show that the extrac-

tion method has good precision (RSD < 4%).

The HPLC conditions used for this study effectively separate phenytoin and dexamethasone. Calibration curves for phenytoin and dexamethasone show excellent linearity ($r^2 > 0.99$). Analysis was performed in less than 11 minutes in an isocratic mode on a reversed-phase C18 column using a mobile phase that is composed of 0.001 M KH_2PO_4, acetonitrile, and methanol adjusted to pH 5.6 (48:32:20).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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