

A MODIFIED QUECHERS EXTRACTION FOR THE DETERMINATION OF DEXAMETASONE

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Abstract

This article describes a modified QuEChERS extraction for dexamethasone (DXM) in commercial and herbal medicines, and a determination was performed by HPLC-UV method. A QuEChERS was optimized using solvent extraction with acetonitrile in the presence of MgSO₄ (500 mg) and NaCl (125 mg). An optimal mobile phase was a mixture of 1% acetic acid and methanol (with ratio 40:60) at a flow rate 1.0 ml.min⁻¹, and UV detection was set at 254 nm. The LOD and LOQ were found as 0.3 and 1.0 ppm, respectively. A method showed adequate precision with %RSD less than 10%. An accuracy was analyzed by adding standard solutions to a herbal pill, and a satisfied recoveries was obtained (100.0±10%).

Keywords: QuEChERS, Steroid, Dexamethasone

Introduction

Dexamethasone (DXM), a cortisteriod, is used as pharmaceutical drug to treat many diseases. It provides a fast accelerate and effective treatment for the anti-inflammatory, autoimmune effects, glucocorticoid resistance, some cancers (hematological malignancies) and muscle diseases. However, it is on a list of the most important medication needed in which a limited amount in the composition is regulated. Due to our body or immune system can be affected by steroids taken. In Thailand, the traditional and commercial herbal medicines are also increase being use to treat and prevent many diseases because of people believe herbal are safe and free from side effect. However, some of medicines are also found steroids over limit in the composition. It may be not controlling well for the production. Then, the amount steroids have to be investigated and monitored. An accurate and effective analytical method to determine is therefore very importance. An effective method has been accomplished when sample preparation is to be

carefully obtained because of most of samples are in matrices. The liquid-liquid extraction (LLE) and solid phase extraction (SPE) are commonly used for separation of analyte in matrices [Iglesias, 2000]. The disadvantages of these extraction procedures include of large amounts of sample and solvents, long time required for the extraction, while SPE cartridge is quite expensive. The analytical methods using UV-Vis spectroscopy [Singh, 2008; Rangaraju, 2013; Pokiya, 2014], high performance liquid chromatography [Gallego, 2002; Urban, 2009; Shadoul, 2011; Prakash, 2012; Desai, 2013] and Gas chromatography [Wasch, 2001; Hidalgo, 2003] have been published.

A QuEChERS extraction is abbreviated from Quick, Easy, Cheap, Effective, Rugged and Safe extraction. It has been accepted for extraction of residual pesticides in vegetables and fruits [Lehotay, 2007; Lucas, 2013; Koesukwiwat, 2010; Lehotay, 2010]. Its extraction steps contains extraction with organic solvent as 1-10 ml in the presence of anhydrous salts and clean up using dispersive-SPE sorbent. Each step is easy performed by centrifuging for few minutes, and a filtrate can be directly analyzed after cleaning. The advantages of QuEChERS include small amounts of sample and solvent and decreasing the time spent to prepare sample. This extraction also takes the advantages of a high efficiency, its standard protocol has been then accepted as AOAC Official Method 2007.1 acetate buffer [Lehotay, 2005] and CEN 15162 citrate buffer. To our knowledge, few works based on a QuEChERS in medicines have been reported, for example as Ref Usui, 2012. Therefore, the work was modified a QuChERS extraction for determination of DXM in commercial and herbal medicines.

Experimental

Chemicals

Most of chemicals: Sodium chloride, acetic acid were obtained from Merck (Germany) all with AR grade. Dexamethasone (DXM) was purchased from Sigma-Aldrich (USA). Acetonitrile, acetone and methanol (HPLC grade) were obtained from Merck (Germany). Magnesium sulfate anhydrous was from Ajax Finechem (USA).

Sample preparations

The commercial and herbal medicine samples were purchased from a drugstore in Bangkok, Thailand. Two types of DXM medicines were used: First, a commercial medicine is a vanishing cream, and second is a herbal pill. A vanishing cream was directly weighted, while a herbal pill was grinded to a small piece. A 0.1 mg of each sample was filled into the tube for QuEChERS extraction.

QuEChERS extraction

A 0.1 mg of sample was added into the centrifuged tube. Subsequently, 5.0 ml of acetonitrile was added. Then, the anhydrous salts: magnesium sulfate anhydrous (500 mg) and sodium chloride (125 mg) were filled into the tube. The mixture was shaken for 5 min and centrifuged at 2000 rpm for 1 min. A supernatant was taken into vial and later filtered using 0.45 μm nylon membrane before injected to HPLC system. The cleaning up with SPE tube could be neglected due to they have no significant between cleaning and un-cleaning. So, the matrices have no interfere to the analyte in this case. LLE was also performed using 1.0 g of each sample and 50.0 ml of organic solvent was added. A solution was later filtered using 0.45 μm nylon membrane before injected to HPLC system.

HPLC conditions

Chromatographic determination was carried out on the Waters HPLC system (Waters w6007172424) equipped with UV-Vis detector (Waters 2489). The detection was set at 254 nm. The HPLC conditions were done under optimal conditions using C₁₈ Waters column (150 mm \times 4.6 mm I.D., 5 μm) with a 1.0 ml.min⁻¹ flow rate. The mobile phase was run using an isocratic elution of 1% acetic acid : methanol with a ratio of 40:60. All samples and solutions were injected at 10 μl . The retention time of DXM was obtained at 7.3 min under these optimal conditions.

Results and discussion

The QuChERS extraction

Compared 3 organic solvents (acetonitrile, methanol and acetone), a QuChERS extraction has been accomplished using acetonitrile extraction. Due to it is easy to dissolve sample, to obtain a clear solution and to achieved the highest peak area. MgSO₄ and NaCl are used to induce phase separation of water and organic phases, where NaCl adding leads to an increase extraction efficiency of polar compound. Weight of MgSO₄ and NaCl with an optimal weight ratio of 4:1 (w/w) was used following the Ref Klinsunthorn, 2011. The amount of MgSO₄ : NaCl were varied at 600 : 150, 500 : 125 and 400 : 100 mg. We obtained 500 mg MgSO₄ and 125 mg NaCl were optimal amount which no excess of anhydrous salt was left.

The reversed phase HPLC condition was investigated which the different types (water : acetonitrile, water : methanol and 1% acetic acid : methanol) and various ratios of the mobile phases. We accomplished of the determination using 1% acetic acid: methanol with a ratio 40:60 at a flow rate 1 ml.min⁻¹ and UV detection at 254 nm. The retention time of DXM was presented at 7.3 min as Fig 1a. The chromatograms of DXM in vanishing cream with different extraction solvents were demonstrated in Fig 1b-1d.

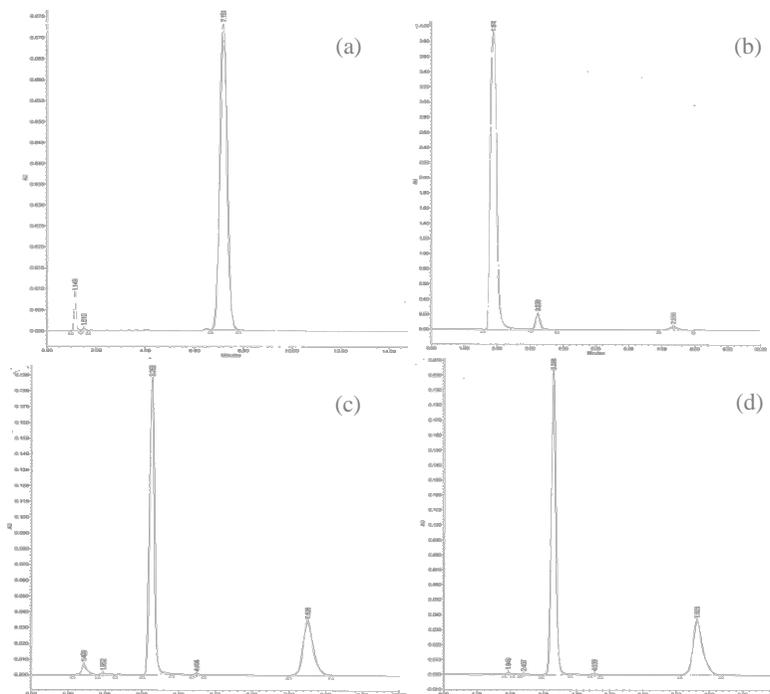


Fig 1. Chromatograms of (a) DXM 100.0 ppm, (b) vanishing cream in acetone extraction, (c) vanishing cream in methanol extraction, (d) vanishing cream in acetonitrile extraction

The method validation

The linearity was studied in the range of 1.0-100.0 ppm. The linear regression of $y = 12835x + 49669c$ was achieved with the R^2 value more than 0.99 ($R^2 = 0.9927$). The LOD (3 signal to noise) and LOQ (10 signal to noise) were 0.3 and 1.0 ppm, respectively. The precision was presented in term of repeatability and reproducibility. A 100.0 ppm of DXM was obtained from 10 batches for intraday-variation, while that for interday-variation was evaluated for 4 days. Results of the repeatability values were acceptable (%RSD < 10%). The accepted percentage of accuracy (96.0%) was obtained by determining the DXM in a vanishing cream compared to its label (5.0 mg DXM).

Application to a traditional herbal pill

The method was applied to analyze DXM in a traditional herbal pill using acetonitrile solvent for a QuChERS extraction in the presence of salt as 500 mg $MgSO_4$ and 125 mg NaCl. An amount of DXM was found lower LOD and then the standard solutions at 50.0 and 100.0 ppm were decided to add into the samples in order to investigate the recovery. A % recovery was found to be satisfactory with 96.0% and 110.0% at 50.0 and 100.0 ppm, respectively. The chromatograms were for example in Fig 2.

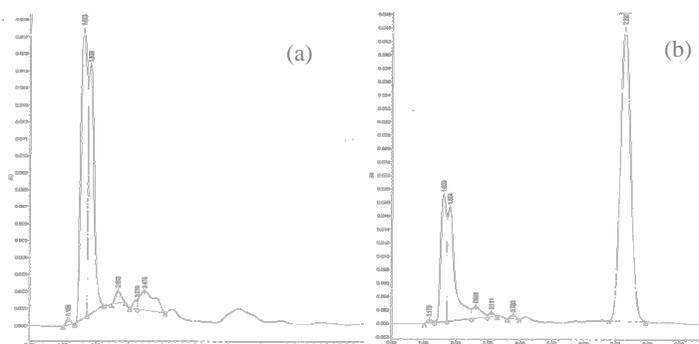


Figure 2. Chromatograms of the herbal pill (a) unspiked solution and (b) spiked solution of 50.0 ppm DXM.

Conclusion

The determination of DXM was achieved using the reverse phase HPLC using in 1% acetic acid : methanol (40:60) condition. In a QuChERS extraction was accomplished under using acetonitrile solvent in the presence of $MgSO_4$ and NaCl. The extraction method can decrease the extraction time and amount of sample and solvent, while the efficiency is acquired compared to LLE extraction. The method was validated where we found the satisfied values.

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