Automated Solid Phase Extraction of Allantoin from Cosmetics and Topical Pharmaceuticals Prior to Analysis by HPLC

Application Note PH0110

An effective automated SPE method was developed for the extraction of allantoin from cosmetic/pharmaceutical products. The use of the Gilson GX-271 ASPEC in combination with CHROMABOND HR-XA SPE cartridges and a NUCLEODUR 100-3 HILIC HPLC column resulted in excellent recovery rates. Use of the Gilson GX-271 ASPEC for automation of the solid phase extraction (SPE) process increased sample throughput, reduced solvent usage and reduced the potential errors that may occur in during manual processing of samples. Automation also permitted scientists to spend more time planning experiments and developing new methods for the analysis of compounds of interest to the laboratory.

Introduction

Allantoin is a heterocyclic organic compound derived from purine (Figure 1). It is also referred to as Glyoxylic Acid Diureide or 5-Ureidhyantoin. Allantoin is a metabolic end product of purine degradation in mammals (with the exception of humans and higher apes), as well as a metabolic intermediate in plants and some bacteria.[1,2]

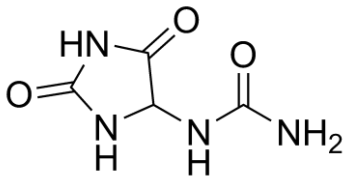

Figure 1. Structure of allantoin (CAS 97-59-6)

Allantoin can be found in many plant species. Comfrey (*Symphytum officinale*) has particularly high levels of this compound. Allantoin has a long history of use in a variety of topical pharmaceuticals and cosmetics for skin care. It is reported to have keratolytic, moisturizing, soothing and anti-irritant properties, and to promote the renewal of epidermal cells and accelerate wound healing.[3] Allantoin is used in pharmaceuticals and dermatologic products in the treatment of ulcers, slow-healing wounds, burns, psoriasis and dry skin.

Recommended levels[4] in cosmetics are from 0.1% to 0.5%. The U.S. Food and Drug Administration (FDA) has classified allantoin as a Category 1 (Safe and Effective) active ingredient for skin protection[5] at use levels of 0.5% to 2.0%. There is a great deal of interest from pharmaceutical and cosmetic manufacturers in determining the amount of allantoin in a variety of products. This application note describes an automated SPE method for the extraction of

Gilson, Inc.
Middleton, WI 53562 USA
Telephone: 800-445-7661

allantoin from a cosmetic product. Allantoin was extracted from a cosmetic product using the automated Gilson GX-271 ASPEC™ System (Figure 2). Allantoin levels were determined using HPLC and a NUCLEODUR® 100-3 HILIC column.

Figure 2. Gilson GX-271 ASPEC™ System

Materials & Methods

SPE Materials

All solvents used were HPLC grade. All reagents were ACS grade or better. Allantoin (>98% purity) was obtained from Fluka Analytical (part no. 05670). Macherey-Nagel CHROMABOND®HR-XA cartridges, 60mg/3mL (Part no. 730950) were used to extract the allantoin from the cosmetic product. The cosmetic products were obtained from a cosmetic manufacturer in Europe. One product had an unknown amount of allantoin. The second product was allantoin free. The allantoin free product was spiked with 5 mg of allantoin.

Preparation of Sample prior to SPE and Additional Liquid Handling Steps

One gram of allantoin sample was mixed with 100 mL of ultra-pure water.

Automated Solid Phase Extraction

The SPE procedure used 60 mg/ 3mL CHROMABOND®HR-XA solid phase extraction cartridges (Macherey-Nagel, Germany). The cartridges were sealed using Gilson 3 mL Sealing Caps.

The solid phase extraction and liquid handling protocol is entirely automated using the Gilson GX-271 ASPEC system.

The SPE steps are summarized with the general schematic provided in the GX-271 ASPEC control software, TRILUTION LH (Figure 3).

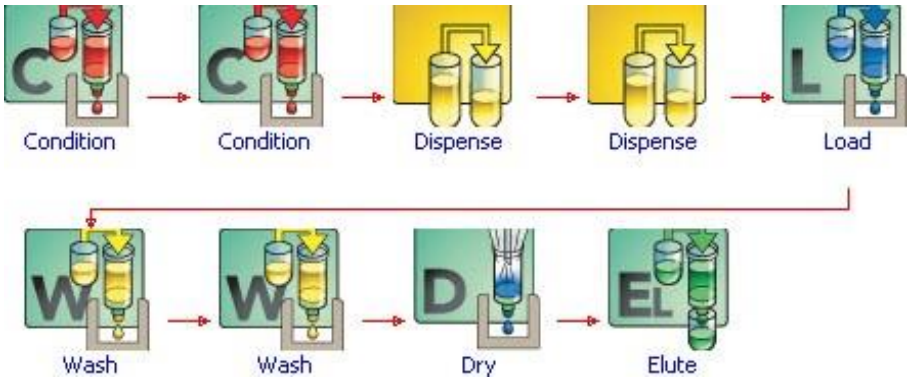

Figure 3. TRILUTION LH Basic SPE Tasks for Solid Phase Extraction of Allantoin from a Cosmetic Product

Gilson, Inc.
Middleton, WI 53562 USA
Telephone: 800-445-7661

The summary of each step are as follows:

1. Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack (Figure 4)
2. Condition the cartridge with 1 mL of methanol at 0.5 mL/min
3. Condition the cartridge with 1 mL of ammonia, w(NH3) = 5% at 0.5 mL/min
4. Dispense 4 mL of sample(1g in 100 mL water) into a tube at 5 mL/min
5. Dispense 400 µL ammonia, w(NH3) = 26% at 0.5 mL/min into the same tube as step above
6. Load 1.1 mL of the sample mix created above onto the SPE cartridge at 0.5 mL/min
7. Wash cartridge with 1 mL of ammonia, w(NH3) = 5% at 0.5 mL/min
8. Wash cartridge with 1 mL of methanol at 0.5 mL/min
9. Dry with 5 mL air, 3 mL/min
10. Move the Gilson Mobile SPE Rack over the collection tubes
11. Elute with 2X 600uL Hydrochloric acid, HCl, 0.1 mol/L at 0.5 mL/min
12. Eluent can be injected directly into the HPLC system

HPLC Analysis

Allantoin concentrations in the extracts were analyzed using high-performance liquid chromatography (Dionex P680, USA) with UV detection (Dionex UVD 17U, USA) using the following conditions:

Column:

Machery-Nagel EC 125/3 NUCLEODUR® 100—3 HILIC (Oart no. 760531.30)

Conditions:

Eluent A: 10 mmol/L Ammonium chloride, pH 3.0) 20
Eluent B: Acetonitrile 80
Flow Rate: 0.3 mL/min
Temperature: Ambient
Injection Volume: 20 µL
Concentration: β(Allantoin) = 5 µg/mL Eluent

Detection:

UV, 214 nm

Results and Discussion

Allantoin is used in a variety of cosmetic products such as skin creams, lip-care products, powders, suntan and sunburn lotions, hair care products, diaper rash ointments and mouthwashes. As shown in Figure 4, recovery of allantoin from the cosmetic product (n=3) was 85.5%

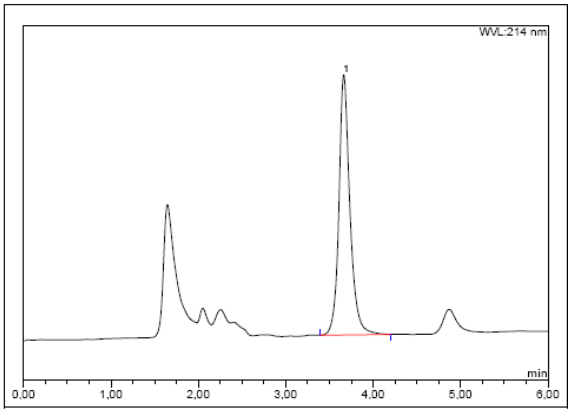

Figure 4. Chromatogram of Allantoin from Cosmetic Product Following SPE Purification. The Retention time from Allantoin is 3.66 minutes.

References

1. Young, E.G., Wentworth, H.P. and Hawkins, W.W. (1944). The Absorption and Excretion of Allantoin in Mammals. *J. Pharmacol. Experi. Therapeutics* **81**: 1-9.
2. Fujiwara, S. And Noguchi, T. (1995). Degradation of Purines: Only Ureidoglycollate Lyase Out of Four Allantoin-degrading Enzymes is Present in Mammals. *The Biochemical Journal* **312** (Part 1):315-18.
3. AKEMA srl (an allantoin manufacturer) website (2008). Allantoin_CFTA.doc. 27/02/2008 Version 1. http://www.akema.it/allantoin.htm
4. Thornfeldt, C. (2005). Cosmeceuticals Containing Herbs: Fact, Fiction or Future. *Dermatologic Surgery* **31** (7 part 2): 873-80.
5. Federal Register (1983, 1990). FDA Monograph on Skin Protectant Drug Products for Over-the Counter (OTC) Human Use. Volume **48**, No. 32, pp. 6820-33 and Volume **55**, No. 11, pp. 25240-81.

Acknowledgments

This study was performed by Dr. Martin Roedel and colleagues at Macherey-Nagel GmbH in Dueren, Germany.

Summary and Conclusions

- An effective automated solid phase extraction (SPE) method was developed for the extraction and quantification of allantoin from cosmetic/pharmaceutical products.
- Automation of the SPE process using the Gilson GX-271 ASPEC™ resulted in increased sample throughput, reduced solvent usage and a reduction in the types of errors that occur in during manual processing of samples.
- Excellent recovery rates (>85%) were achieved using the Gilson GX-271 ASPEC system in combination with CHROMABOND HR-XA SPE cartridges and a NUCLEODUR 100-3 HILIC HPLC column.
- Automation also increased walk-away time, permitting scientists to spend more time planning experiments, analyzing data, and developing new methods.