



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

Keywords: Allantoin, Automation, CHROMABOND® HR-XA, Cosmetics, Glyoxylic Acid Diureide, GX-271 ASPEC™, HILIC, HPLC, Ointments, Pharmaceuticals, Sample Preparation, Solid Phase Extraction, SPE, Topical Pharmaceuticals, 5-Ureidohydantoin

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

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 (Young et al., 1944; Fujiwara et al., 1995). Allantoin can be found in many plant species. Comfrey (*Symphytum officinale*) has particularly high levels of allantoin.

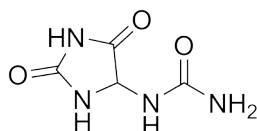


Figure 1. Chemical Structure of Allantoin (CAS no. 97-59-6).

Allantoin has a long history of use in a variety of topical pharmaceuticals and cosmetics for skin care. It has keratolytic, moisturizing, soothing and anti-irritant properties. Allantoin promotes the renewal of epidermal cells and accelerates wound healing (AKEMA, 2008). Allantoin is used in pharmaceuticals and dermatologic products in the treatment of ulcers, slow-healing wounds, burns, psoriasis and dry skin. The U.S. Food and Drug Administration (FDA) has classified allantoin as a Category 1 (Safe and Effective) active ingredient for skin protection (Federal Register, 1983, 1990) at use levels of 0.5% to 2.0%.

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. Recommended levels in cosmetics are from 0.1% to 0.5% (Thornfeldt, C., 2005).

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



Figure 2. Gilson GX-271 ASPEC™ System with 406 Single Syringe Pump (part no. 2614007).

Experimental Conditions

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 Solid Phase Extraction and Additional Liquid Handling Steps

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

Automated Solid Phase Extraction

The Gilson GX-271 ASPEC System was configured as follows:

Description	Part Numbers
GX-271 ASPEC w/ Single 406 Syringe Pump	2614007
10 mL Syringe	25025345
SPE Pressure Reg. Assembly and Plumbing package for gas + 10 mL Plumbing Package	25051376, 2644703, and 2644701
221 x 1.5 x 1.1 BV Tapered Probe and Guide Assembly for 1.5 mm Probes	27067374 and 26046228
Rinse Stations	26034551 and 26034555
Locator Tray for Five 20-series Racks	26041033
DEC Accessory Set for 3 mL SPE Cartridges	2604702
Rack Code 343 for 80 13 x 100mm Tubes	260440025
Rack Code 345 for 44 16 x 150mm Tubes	260440041
Solvent Reservoir Tray Insert for 700 mL bottles and pkg of four 700 mL solvent bottles	260440005 and 543701700
Safety Shield Assembly, GX27X	2604706
TRILUTION® LH Software Package	21063020, 210630R20 and ORACLE10GXE

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).

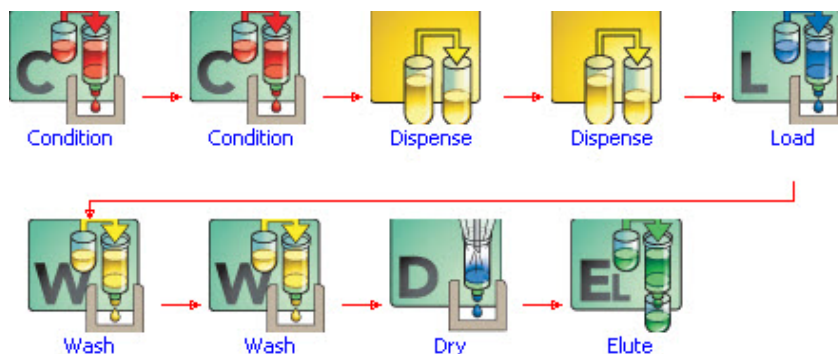


Figure 3. TRILUTION LH Basic SPE Tasks for Solid Phase Extraction of Allantoin from a Cosmetic Product

The summary of each step are as follows:

- Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack (Figure 4)
- Condition the cartridge with 1 mL of methanol at 0.5 mL/min
- Condition the cartridge with 1 mL of ammonia, $w(\text{NH}_3) = 5\%$ at 0.5 mL/min
- Dispense 4 mL of sample (1 g in 100 mL water) into a tube at 5 mL/min
- Dispense 400 μL ammonia, $w(\text{NH}_3) = 26\%$ at 0.5 mL/min into the same tube as step above
- Load 1.1 mL of the sample mix created above onto the SPE cartridge at 0.5 mL/min
- Wash cartridge with 1 mL of ammonia, $w(\text{NH}_3) = 5\%$ at 0.5 mL/min
- Wash cartridge with 1 mL of methanol at 0.5 mL/min
- Dry with 5 mL air, 3 mL/min
- Move the Gilson Mobile SPE Rack over the collection tubes
- Elute with 2X 600 μL Hydrochloric acid, HCl, 0.1 mol/L at 0.5 mL/min
- Eluent can be injected directly into the HPLC system



Figure 4. Gilson Mobile SPE Rack

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: Macherey-Nagel EC 125/3 NUCLEODUR® 100-3 HILIC (Part no. 760 531.30)
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

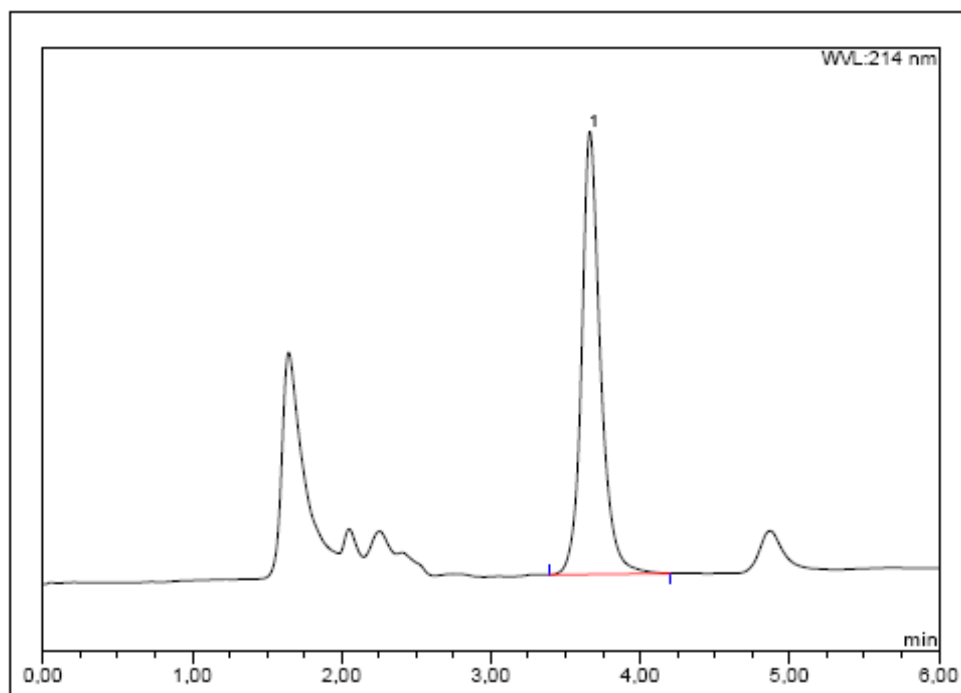


Figure 5. Chromatogram of Allantoin from Cosmetic Product Following SPE Purification. The Retention Time for Allantoin is 3.66 minutes.

The recovery of allantoin from the cosmetic product (n=3) was 85.5%

Conclusion

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.

Using 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 scientific experiments and developing new methods for the analysis of compounds of interest to the laboratory.

References

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