Chromatography Technical Note No AS108

Automating DNPH derivatisation for aldehyde and ketone analysis

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Introduction

Airborne aldehydes and ketones are collected by passing air through a cartridge containing 2,4-dinitrophenylhydrazine (DNPH). Carbonyl compounds react with the DNPH to form derivatives in the form of hydrazones which are then immobilised on the cartridge. Figure 1 shows the derivatisation of aldehydes and ketones using DNPH.

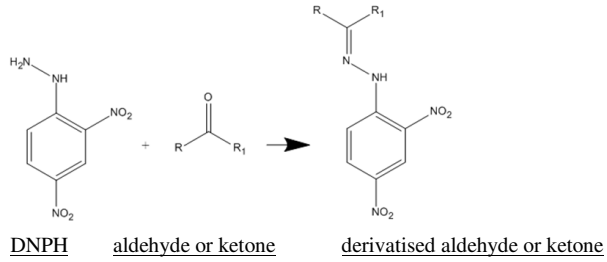

Figure 1 - Reaction of DNPH with aldehydes or ketones.

These compounds can be easily eluted from the cartridge with acetonitrile and analysed by HPLC with UV detection.

Manual Process

After the required amount of air (to be tested) has been passed through the DNPH cartridge, a syringe containing acetonitrile can then be attached. Acetonitrile is used to elute the derivatised aldehyde or ketone which has been trapped on the DNPH cartridge. The extract is collected into a 5 ml volumetric flask and the flow of acetonitrile is stopped when 5 ml has been collected. This is performed by eye, checking that the top of the meniscus has reached the 5ml mark. The volume of extract collected is critical as the concentration of the derivatised product will dependent on the volume collected. The extract is then shaken to ensure that the solution is adequately mixed, and a portion of this solution is pipetted into a vial for HPLC analysis by UV detection.

Automation of the process

To automate this method, a good seal is required between the DNPH cartridge and the injection needle from the syringe attached to the MultiPurpose Sampler (MPS). This will allow delivery of the solvent through the cartridge. Figure 2 shows a picture of the DNPH cartridge with sealing unit (transport adaptor) and a 0.2 micron filter. Figure 3 shows a schematic diagram indicating a good seal between the needle and DNPH cartridge.

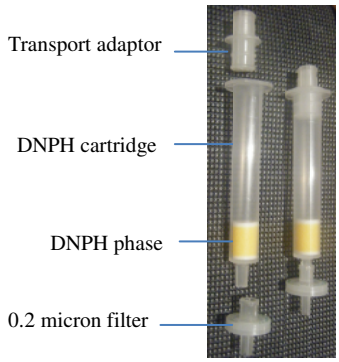

Figure 2 - Picture of DNPH cartridge with sealing unit (transport adaptor) and 0.2 micron filter.

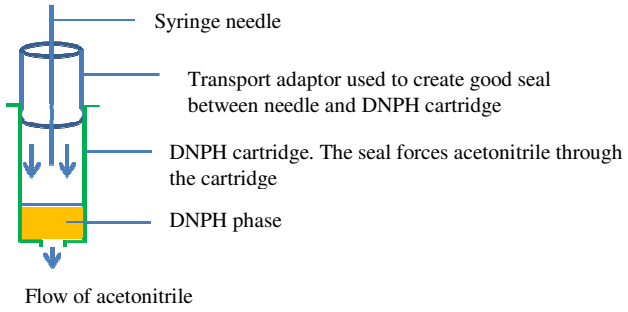

Figure 3 - Schematic view of the sealing process.

Instrumentation

Gerstel MultiPurpose Sampler MPS 2 XL (dual head)
Maestro Version 1.4.8.14/3.5
CTC Analytics Cooled tray
Agilent 1260 Quaternary Pump
Agilent 1260 Diode Array detector
Agilent 1260 Column Oven
Anatune 300 Automated DNPH unit*

*under development

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Figure 4 shows a picture of the automated DNPH unit which is under development at Anatune. For each extraction, the MPS head will pull the top drawer forward so that the cartridge is in line with an empty 10 ml vial which is situated on the bottom shelf.

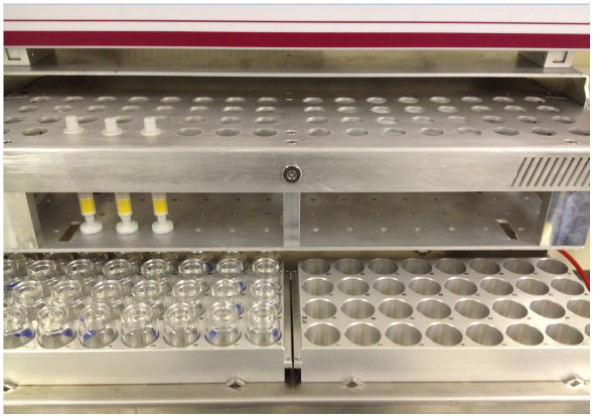

Figure 4 - Automated DNPH unit (Anatune 300).

Method

From previous experimental work, it has been found that the addition of 5.6 ml of acetonitrile to the cartridge will produce a 5 ml extract. Figure 5 shows the photo of how the system will operate to automate the DNPH process.

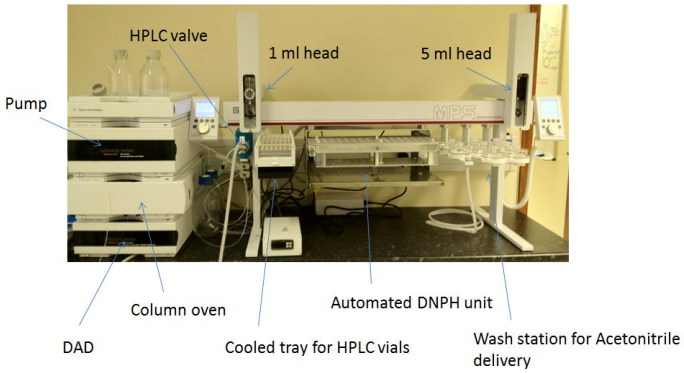

Figure 5 – Automated DNPH MPS system.

After the DNPH cartridge has been aligned with an empty 10 ml vial, 5.6 ml of acetonitrile is added to the cartridge using a 5 ml syringe on an MPS head. An air push through the cartridge is then performed. This is to maximize the amount of extract produced. The extract is then mixed and a 1ml aliquot (using a 1 ml syringe) is then added to a sealed 2 ml HPLC vial which is seated within the cooled tray which is subsequently injected onto the HPLC. Up to 64 extractions can be automated.

For this study, 20 automated extractions were performed. Each extraction uses new transport adaptor, new SPE cartridge (LpDNPH 3ml 350 mg Supelco) and a new 0.2 micron filter (Nylon, Sigma Aldrich). 5.6 ml of acetonitrile was added to each cartridge.

Results

Table 1 shows the volume of acetonitrile collected for 20 different automated extractions.

Description	Volume (ml)
Extraction 1	5.00
Extraction 2	5.00
Extraction 3	5.00
Extraction 4	4.90
Extraction 5	4.85
Extraction 6	4.95
Extraction 7	4.95
Extraction 8	4.95
Extraction 9	4.90
Extraction 10	4.90
Extraction 11	4.85
Extraction 12	4.95
Extraction 13	4.70
Extraction 14	4.95
Extraction 15	4.95
Extraction 16	4.95
Extraction 17	5.00
Extraction 18	4.95
Extraction 19	5.00
Extraction 20	5.00
Mean	**4.94**
sd	**0.07**
CV	**1.47**

Table 1 - Volume of acetonitrile collected for 20 automated extractions.

Discussion

Automating this extraction will drastically improve productivity by freeing up manual labour associated with this technique and will hopefully improve any potential experimental errors associated with the method. With the Prep-Ahead function, in the Maestro software, the automated sample preparation can be incorporated within the HPLC Run time which is 12.5 minutes long.

The new system is capable of automating up to 64 DNPH extractions and will shortly be installed at Reckitt Benckiser for aldehyde and ketone analysis in Hull.

I would like to thank Vicki Morris at Reckitt Benckiser for allowing Anatune to publicize their new purchase which includes the new automated DNPH unit (Anatune 300).

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