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Analysis of a residual diamine in a pharmaceutical polymer using solid phase extraction with analysis by gas chromatography mass spectrometry

D.G. Watson [a,*], L. Li Xin [a], J.M. Midgley [a], D. Carr [b]

[a] *Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G4 0NR, UK*
[b] *Controlled Therapeutics (Scotland), Peel Park Campus, East Kilbride, G74 5PB, UK*

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Abstract

A method was developed for the analysis of 4,4′-methylenebiscyclohexylamine (DMDA) and 4,4′-methylenedicyclohexylisocyanate (DMDI) in a pharmaceutical polymer. The DMDA was extracted from the polymer with either buffer (0.1 M potassium phosphate pH 3.1) and the extract was passed through a SCX solid phase extraction cartridge. It was eluted from the cartridge with methanolic ammonia and then converted to its heptafluorobutyramide (HFB) derivative prior to analysis by gas chromatography–negative chemical ionisation mass spectrometry (GC–MS) in the negative ion chemical ionisation (NICI) mode. It was not possible to directly measure DMDI and it was thus analysed by selecting extraction conditions such that it would decompose to DMDA. The quantification of the residues in the polymer was based on the method of standard additions since this gave a better indication of the recovery from the complex matrix.

Keywords: Polymer; Residues; Solid phase extraction; Gas chromatography–mass spectrometry

1. Introduction

A pharmaceutical polymer invented at the University of Strathclyde [1] and developed by Controlled Therapeutics has been successfully utilised as a delivery system for Dinoprostone, by means of a retrievable vaginal pessary, for the purposes of cervical ripening in women at or near term. The polymer is based on polyethylene glycol (MN8000) which has been reacted with hexanetriol (crosslinking agent) and 4,4′-methylenedicyclohexylisocyanate (DMDI)—a chain extender. There is a strict stoichiometric balance between the isocyanate of DMDI and the hydroxyl groups on PEG 8000 and hexanetriol. DMDI is widely used as crosslinking agent in polymer synthesis. The commercially available compound is synthesised by hydrogenation of 4,4′-methylenediphenylisocyanate (MDI) and is thus a mixture of isomers. There is less information available on the toxicity of DMDI than there

* Corresponding author. Tel.: +44-141-5482651; fax: +44-141-5526443; e-mail: d.g.watson@strath.ac.uk.

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is for MDI. Like all common industrial isocyanates, there is threshold limit value set at 0.005 mg m^{-3} in air for 8 h exposure [2] and at higher levels it can cause sensitivity reactions by inhalation. The oral LD50 (in rats) is reported to be 9900 mg kg^{-1}, which suggests by oral absorption it presents a significantly lower risk. DMDI breaks down, upon exposure to moisture, to 4,4′-methylenedicyclohexylamine (DMDA). This compound has been found to be non-mutagenic in *Salmonella* mutagencity tests [3].

MDI slowly hydrolyses, upon exposure to moisture, to yield 4,4′-methylenedianiline (MDA). A number of papers have been published concerning the measurement of MDA in the urine of industrial workers as a means of monitoring the level of exposure to MDI in the industrial environment [4–7]. Gas chromatography–negative chemical ionisation mass spectrometry (GC–MS) is the method of choice for the determination of MDA, because of its high sensitivity. Preparation for GC–MS analysis involves derivatisation of the MDA to either its dipentafluoropropionyl [3–5] or its diheptafluorobutyryl [6] derivative.

In the current work a method based on GC–MS in the negative ion chemical ionisation (NICI) mode has been developed for the measurement of DMDA and DMDI batches of a pharmaceutical polymer. The difficulty of this type of analysis arises from the complexity of the matrix from which the trace residues are being extracted which leads to variable recovery of the residue and interference by other trace components extracted from the matrix. To reduce these problems, the method of standard additions was used to in combination with solid phase extraction (SPE) in order to develop a limit test for crosslinking residues in the insoluble polymer.

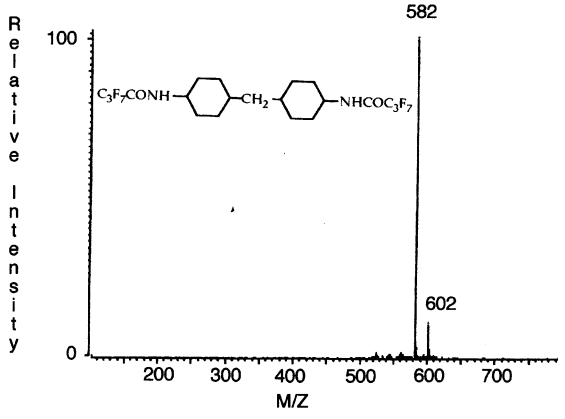

Fig. 1. Mass spectrum of DMDA diHFB derivative under negative ion chemical ionisation (NICI) conditions.

2. Materials and methods

2.1. Chemicals

Chemicals were obtained from the following sources: 4,4′-methylenedicyclohexylamine (DMDA), 1,12-diaminododecane (DADD), 4,4′-methylenedicylohexylisocyanate (DMDI), and heptafluorobutyric anhydride (HFBA) from Aldrich Chemicals, Dorset, UK; methanol HPLC grade, ethyl acetate HPLC grade, and water HPLC grade from Rathburn Chemicals, Peebleshire, UK; toluene from AR BDH, Dorset, UK; Isolute SCX SPE cartridges (100 mg) from Crawford Scientific, Lanarkshire, UK.

2.2. Preparation of SCX columns for extraction

The SCX column was washed with methanol (10 ml), water (10 ml) and finally potassium phosphate buffer (10 ml, pH 3.1, 0.1 M). The column was then ready for application of the sample.

2.3. Sample processing

2.3.1. Procedure 1: standard additions of 4,4′-methylemebiscyclohexylamine to the polymer

Samples ($7 \times \approx 1.0$ g) of chopped polymer material were weighed out and then phosphate buffer (40 ml, pH 3.1, 0.1 M) was added. To each sample the DADD internal standard (20 ng in 20 μl of acetonitrile) was added with varying amounts of DMDA (2×0, 4, 8, 16, 32, and 64 ng in 20 μl of acetonitrile). The polymer samples were left to swell (1 h at room temperature) and were then transferred, one at a time, to a Waring blender and homogenised. Portions (20 ml) were taken from each sample and then applied to SCX

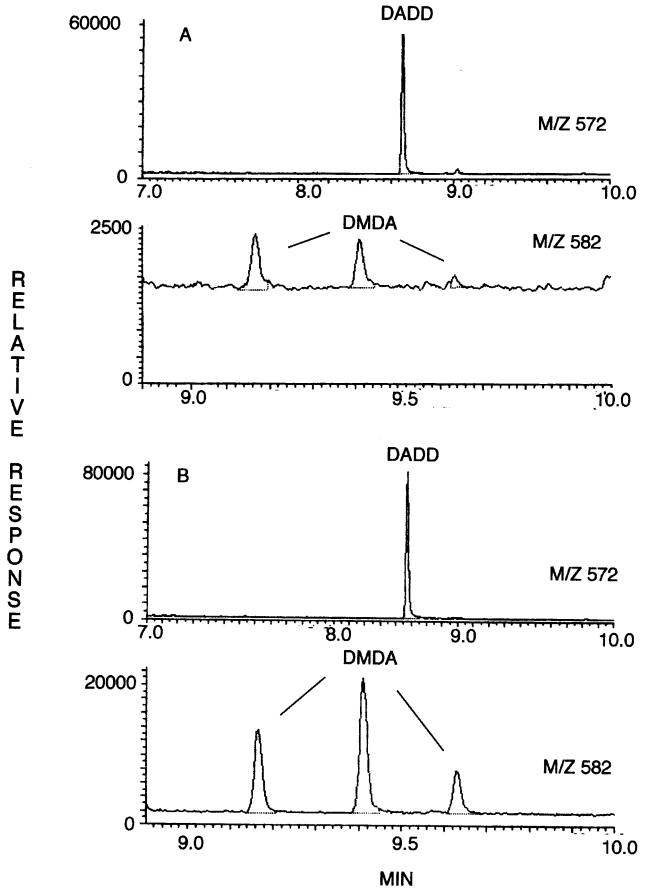

Fig. 2. A, SIM trace of diHFB derivative of extract from 1 g of polymer with 20 ng of DADD internal standard included in extraction buffer. B, SIM trace of diHFB derivative an extract from the same batch of polymer with 16 ng of DMDA and 20 ng of DADD included in the extraction buffer.

solid phase extraction columns attached to a vacuum manifold. The columns were washed with water (10 ml) and then eluted with methanolic ammonia (2 M, 2 ml). The methanolic ammonia solution was evaporated under a stream of nitrogen, the residue was reacted with HFBA (50 μl) at 60°C for 15 min. Toluene (0.5 ml) was added and the solution was then shaken with potassium phosphate buffer (0.5 ml, 1 M, pH 7.0). The toluene layer was removed and was evaporated to dryness under a stream of nitrogen. Finally toluene (50 μl) was added to dissolve the residue. The samples were then ready for analysis by GC–MS.

2.3.2. Procedure 2: standard additions of 4,4′-methylenedicyclohexylisocyanate to the polymer

Samples ($7 \times \approx 1.0$ g) of chopped polymer material were weighed out and phosphate buffer (40 ml, pH 3.1, 0.1 M) was added. To each sample the DADD internal standard (20 ng in 20 μl of methanol) was added with varying amounts of DMDI (2×0, 4, 8, 16, 32, and 64 ng in 20μl of acetonitrile). The polymer samples were left to swell in the the buffer at 60°C for 1 h and were then transferred to a Waring blendor and homogenised. Thereafter the procedure was as described under procedure 1.

Table 1
Equation of standard additions calibration curves, mean and RSD for residues of DMDA/DMDI in batches of polymer determined from standard additions of DMDA in the range 0–64 ng per sample

Batch no.	Standard additions of DMDA		
		Mean content ng g^{-1}	RSD
1	$y = 0.50 + 0.026x$ $r = 0.998$	19.23	±13.7
2	$y = 0.11 + 0.017x$ $r = 0.999$	5.58	±26.0
3	$y = 0.105 + 0.025x$ $r = 0.999$	4.00	±32.6

Table 3
Equation of standard additions calibration curves, mean and RSD. for residues of DMDA/DMDI in batches of polymer determined from standard additions of DMDI in the range 0–64 ng per sample[a]

Batch no.	Standard additions of DMDI		
		Mean content ng g^{-1}	RSD
1	$y = 0.12 + 0.034x$ $r = 0.996$	18.42	±12.2
2	$y = 0.12 + 0.034x$ $r = 0.996$	3.82	±51.7
3	$y = 0.062 + 0.016x$ $r = 0.997$	3.62	±35.3

[a] The DMDI was measured as its decomposition product DMDA.

2.4. Instrumentation

A Hewlett-Packard HP5988A GC–MS system was used in the negative chemical ionisation mode with methane as the reagent gas. The GC was fitted with a HP-1 column (12 m × 0.2 mm × 0.33 μm film) with helium as a carrier gas at 5 p.s.i. The oven was programmed as follows 100°C (1 min) then 10°C min^{-1} to 300°C. The mass spectrometer was then used to monitor ions at m/z 572 for the $[M\text{-}HF]^-$ ion of the diHFBA derivative of DADD and 582 for the $[M\text{-}HF]^-$ ion of diHFBA derivative of DMDA.

2.5. Statistics

The standard deviations for the standard additions methods were calculated according to standard procedures [8]. The relative standard deviation (RSD) is given for 95% confidence limits.

Table 2
Conditions tested for promoting decomposition of DMDI

Decompostion conditions for 20 ng of DMDI	Area of DMDA/area of 20 ng DADD
DMDI in buffer at room temperature for 1 h	0.5023
DMDI in buffer at buffer 60°C for 1 h	0.7209
DMDI in buffer 100°C for 1 h	0.7435
DMDI in buffer 150°C for 1 h	0.5538

3. Results

Fig. 1 shows the mass spectrum obtained under NICI conditions for the diHFB derivative of DMDA, most of the ion current in the spectrum is carried by the $[M\text{-}HF]^-$ ion. The mass spectrum of the diHFB derivative of the DADD internal standard was similarly dominated by the $[M\text{-}HF]^-$ ion. When selected ion monitoring was carried out the limits of detection for the DMBA were ≈ 40 pg on column, and since the extracts were quite 'clean' following SPE it should be possible to inject the entire sample on column if required, thus improving the limit of detection.

Table 4
Measurement of DMDA and DMDI as DMDA in a polyethylene glycol based polymer

Batch no.	Mean content of DMDA (ng g^{-1})	RSD
1	18.92	3.4
2	2.77	9.7
3	2.15	7.2

Fig. 2A shows a selected ion monitoring (SIM) trace for residual DMDA (m/z 582) extracted from ≈ 1 g of polymer in comparison with 20 ng of DADD (m/z 572) added prior to buffer extraction. Fig. 2B shows DMDA extracted from ≈ 1 g of the same batch of polymer to where 16 ng of DMDA had been added to the extraction buffer. The DMDA produces three chromatographic peaks, this is due to the fact that commercial product is a mixture of stereoisomers resulting from the preparation of DMDI by non-stereospecific reduction of methylenediphenylisocyanate.

The results of standard additions of DMDA to three batches of polymer are shown in Table 1. The method was linear and the precision acceptable for analysis of such a low amount of analyte particularly when it is considered that the highest precision for a standard additions method is when $y = y$ [8] and that the intercept representing the amount of DMDA extracted from the polymer is well below y.

In order to determine whether there was any undecomposed DMDI in the polymer extraction procedure 2 utilising standard additions were carried out. The conditions tested for promotion of DMDI decomposition to DMDA are given in Table 2. Heating the polymer at 60°C for 1 h in buffer were selected as being suitable for promoting DMDI decomposition to DMDA. Under these conditions the recovery of the decomposed DMDI was uniform with the standard additions curve being linear over the range 0–64 ng. The results obtained from standard additions of DMDI to three batches of polymer are given in Table 3. The method developed does not distinguish between DMDA and DMDI residues but it is probable, in view of its instability, that little DMDI survives in the polymer matrix. The data reported in Table 2 demonstrates that even a relatively short exposure to moisture at room temperature results in decomposition of DMDI to DMDA. Consequently the results reported in Table 1 reflect the sum of DMDA and a contribution from any DMDI present in the polymer matrix. Comparison of the mean contents of DMDA/DMDI determined in the three batches of polymer show good agreement between procedures 1 and 2.

To complete the study and develop a method which could ultimately be used as a limit test the following procedure was used. Five samples (1 g) from each of the three batches of polymer were treated as described in procedure 2. DMDI was, however, not added to any of these samples. The results from the three batches were compared with two samples (1 g) from each of the three batches of polymer to which 20 ng of DMDI had been added. These samples were also treated as described in procedure 2. The mean amounts of DMDI determined as DMDA for each batch of polymer are reported in Table 4. The amount of DMDI as DMDA found in batch 1 is very close to that determined by the standard additions method, the amounts of DMDA found in batches 2 and 3 are slightly lower than those determined from the standard additions method. The determination against one point gave much better precision than the method of standard additions which is as expected from statistical considerations [8].

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