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Development of an analytical method for the evaluation of *N,N*-dimethylformamide in dosage form design

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N,N-Dimethylformamide (DMF) is a well-known chemical entity that is extensively used for pharmaceutical, biomedical and chemical applications. Previous research identified the need for the development of an effective dosage form for the systemic delivery of DMF due to its unique antiviral properties. For purposes of quality control and evaluation during pharmaceutical product development, development of an analytical method was required. A gas chromatographic (GC) method was developed with a flame-ionization detector (FID) on a carbowax packed glass column. 2-Methoxyethanol was used as internal standard. The analytical method proved to be capable of separating DMF and 2-methoxyethanol adequately within a relatively short runtime of 2.5 min. The analytical method described was primarily developed for use in dissolution studies of DMF containing delivery systems. Various physicochemical properties of candidate internal standard materials were correlated with the observed retention times of these compounds. The best correlation ($r^2 = 0.8077$) was obtained between the boiling point and the retention time of the compounds for the current application. The boiling point of an internal standard candidate material may therefore be useful in predicting the retention time of that compound under similar conditions.

1. Introduction

N,N-Dimethylformamide (DMF) is a well-known chemical entity with several applications within the pharmaceutical, biomedical and chemical fields. Previous research identified the therapeutic potential of DMF due to its antiviral properties. Numerous studies have been conducted to investigate the anti-viral effect of polar compounds, in particular cell differentiators. Most of these studies were *in vitro* studies and the agents investigated were dimethylsulfoxide (DMSO), DMF and other polar substances (Viza et al. 1989, 1991, 1992).

DMF is extensively used in the manufacturing of plastic, acrylonitrile fibres, synthetic leather, pharmaceuticals, dyes, and in the synthesis of feedstock. It is also used as an absorbent for gasses, a selective extractant, a solvent for electrolytes, in recrystallization and purification and as a reaction medium (BASF 1989). Due to the industrial importance of DMF and the number of workers exposed to it, numerous studies have been conducted on its toxicity and various other physicochemical properties (Matheson et al. 1979; Kestell et al. 1987; Kawai et al. 1992; Trevisani et al. 1993; Augustyns et al. 1998; Wrbitzky 1999).

DMF, DMSO and pyridine-*N*-oxide (PNO), proved to be powerful inhibitors of human immunodeficiency virus (HIV) production by the infected cell. All three compounds produced a significant reduction in HIV production as monitored by assay for reverse transcriptase activity and/or amount of viral core antigen (p24) in the supernatant. This strengthened the theory that HIV production may be dependent on the differentiation state of the host cell. These compounds reduced viral production indirectly and they did not have any direct effect on the virus or on the modification of T-helper cells as defined by trans-membrane glycoprotein CD4 receptors. The results obtained in these studies prompted the authors to include a motivation for an *in vivo* HIV investigation of these compounds for the clinical management of acquired immunodeficiency syndrome (AIDS) (Viza et al. 1989). Therefore, a suitable analytical method needed to be developed.

Due to the relatively low boiling point of DMF and being a liquid at room temperature, it is a good candidate for gas-chromatographic (GC) analysis. Various examples of analytical methods used to analyze DMF published in the literature are listed in Table 1.

The published methods were mainly used to analyze DMF in biological fluids (urine and blood) and during occupational exposure monitoring. Extraction steps from the body fluids were extensively used during these applications. The current application required the analysis of small water based samples preferably without extraction steps, as the extraction efficacy would require time-consuming validations with increased costs. A controlled release dosage form was chosen as the most suitable delivery system for DMF due to its relatively short biological half-life ($t_{1/2} = 1$ to 2.3 h) (Hundley et al. 1993) and for

Table 1: Summary of published analytical methods for DMF (NIOSH 1991)

Analytical Method	Application	Reference
GC	Pure DMF	BASF (1989)
GLC	Vapour	NIOSH (1991)
GC	Urine	Barnes and Henry (1974)
GC	Urine	Kimmerle and Eben (1975)
GC	Urine	Krivanek et al. (1978)
GC	Urine	Mráz and Tureček (1987)
GC	Urine	Lauwerys et al. (1980)
GC	Blood	Kimmerle and Eben (1975)
GC	Blood	Lundberg et al. (1983)
GC	Blood, liver, kidney, brain, adrenals	Hundley et al. (1993)

this reason dissolution studies need to be accurately done in order to closely monitor the release of DMF over extended periods of time. Dissolution studies were conducted using a Valia-Chien diffusion cell system, resulting in samples for analysis with a volume ranging between 100 and 200 µl. These samples had to be analyzed without further dilution and the internal standard had to be added to the sample by standard addition in order to obtain results withing the working range of the FID.

2. Investigations, results and discussion

Injection of DMF samples without the internal standard yielded small and inconsistent peaks at approximately the same retention time as that of the internal standard, isopropanol. Due to the closeness in the retention times, these small peaks sometimes appeared under the peak of the internal standard causing variation in the peak height of the internal standard. At the lower concentration ranges of the dissolution tests, this sometimes caused inconsistent results. The only possible cause for this was a water peak that eluates underneath the peak of the internal standard. Even though it is generally accepted that water does not yield a peak on FID detectors, it is possible for water to ionize to form hydronium ions that could explain the observed event. This aspect warrants further investigation but falls outside the scope of this study.

The only suitable internal standard candidates are those that can be separated from water. These compounds included 2-methoxyethanol, acetic acid, benzyl alcohol, diethylene glycol, dimethyl sulfoxide, dioxane, glycerol, phenol and propylene glycol. Some of these compounds were excluded based on poor detector response and relatively small peak areas, which include acetic acid, benzyl alcohol and glycerol. Diethylene glycol did not produce a peak within a five minute runtime and the phenol peak just started to form at the end of a five minute run. The dioxane had a relatively close retention time to that of water. The use of dimethyl sulfoxide and propylene glycol would have caused an extension of the run from 2.5 to 4.5 or 5 minutes due to the retention times of these compounds. These two compounds also exhibited peak broadening. It was therefore decided to use 2-methoxyethanol as internal because it complied better with the set requirements.

The results obtained for the correlation of physicochemical properties of the internal standard candidate materials and their retention times are included in Table 2.

Table 2: Correlation coefficients for linear and logarithmic fits of the physicochemical properties of candidate internal standard materials as a function of retention time

Physico-chemical property	Linear fit correlation coefficient (r^2)	Logarithmic fit correlation coefficient (r^2)
Molar mass	0.1892	0.2156
Boiling point	0.6534	0.8077
Melting point	0.3636	0.3865
Relative density	0.3254	0.4408
Vapour pressure	0.0105	0.0005
Relative vapour density	0.1977	0.2387
Log P_{ow}	0.0015	0.0102
%C	0.0332	0.011
%H	0.694	0.0937
%O	0.041	0.0218

From the results in Table 2 it is clear that the best correlation between the physicochemical properties of the candidate internal standard material and the retention times obtained was for the boiling point of the material (r^2 for linear fit = 0.6534 and r^2 for logarithmic fit = 0.8077). The logarithmic fit may therefore be useable as a relative predictor of the retention time of a material if the boiling point of the material is known. The oil water partition coefficient or log P is a recommended tool for the development of analytical methods (Lund 1994), however from the above results it becomes clear that under the conditions used for this study this variable is not applicable. The correlations obtained for the other physicochemical properties did not indicate considerable correlations and therefore cannot be used for retention time prediction. The chemical properties of the candidate internal standard materials were included in the correlation determinations to study the effect of C (non-polar) and O and H for possible polar interaction with the carbowax packing material of the column. The results however clearly indicate that these properties are not useable for retention time prediction. These chemical properties may be more applicable to HPLC analysis.

The concentration of the internal standard was adapted in the sample in order to obtain a peak area of approximately the 15% or 17% of the height of the standard peak. This was done to reduce the effect of the tailing observed from the internal standard peak and to ensure that accurate results could the obtained for the DMF peak at the lowest possible concentrations.

A typical chromatogram obtained during analysis of a sample from the primary standard is included in Fig. 1.

3. Experimental

3.1. Materials

The method development was conducted on a Carlo Erba Strumentazione 4200 Gas Chromatograph equipped with an FID. All chemicals used were analytical grade unless otherwise indicated. DMF was obtained from Sigma-Aldrich, South Africa (Fluka brand). Distilled water (Fistream single distiller, Labhouse, South Africa) was used as the carrier system for the analysis. The column used for analysis was a packed Carbowax 20M 80/20 column with potassium hydroxide, 2 meter length (Anatech, South Africa). All samples were injected using a 5 µl SGE (Scientific Glass Engineering Analytical Syringe). Air flow rates were measured with a 7-Gas Flow Meter available from Chromatography Research Supplies, (USA). Integration was achieved by using the PeakSimple Chromatography System (SRI Model 203 single channel serial port on software version no 3.21) connected to a computer using Windows 98® as operating system. Data and graphical analysis were conducted using a Microsoft Excel® spreadsheet. The following primary software settings were used during analysis:

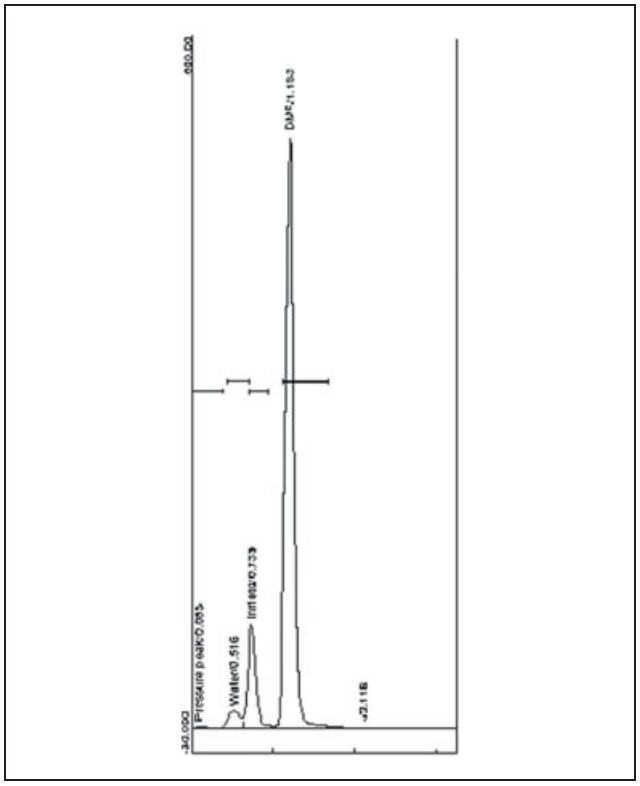

Fig.: Typical chromatogram of a standard generated during the analysis of DMF

Details:	Default Display:	Max	800.00 mV
		Min	−30.000 mV
		End time	2.500 min
	Integration:	Peak	99.00%
		Baseline	100.00%
		Area reject	0.100
	Chart speed	2.54 cm/min	

3.2. Methods

Initially, 1 µl of pure DMF was injected directly into the detector in order to confirm response to DMF. The boiling point of DMF is 153 °C (Budavari 1989) and therefore the initial column temperature was set to 155 °C, with injector and detector each set to a temperature of 200 °C. The above settings were increased in order to optimize the runtime, peak symmetry and to eliminate splitting of the peak of the primary analyte. The final apparatus settings and conditions are reported under apparatus conditions.

Determination of the retention time of the apparent water peak was the next step in the process of method development. The apparent retention time of the water peak was determined by the injection of 1 µl of distilled water onto the column. Any peaks detected were then ascribed to detector response to water. Thereafter, volatile compounds suitable for use on GC were injected in an attempt to identify a compound that could be separated from the water peak without an increase of the total runtime of the analysis. An internal standard was selected from this range of volatile compounds based on its chemical structure, compatibility with the carbowax column, boiling point and water solubility. The different candidate internal standard compounds were individually added to water in a concentration of 10% v/v before injection on the GC. The retention time and the presence of a water peak were noted. The retention times of DMF and water during analysis under the chosen experimental conditions were also determined. The shortest possible runtime with acceptable resolution was the goal of this method development. The final choice and method of addition of the internal standard used in the developed method is reported under results and discussion.

The method was also developed for use on the final product. The method used to determine the assay of the product under development required dilution and sample preparation for this application.

Graphs of the retention times of the different internal standard candidate compounds were plotted as a function of selected phycisochemical properties of the different compounds in order to identify the property of the compounds that provided the best correlation with retention time. The oil/water partition coefficient of a compound is used as a predictor of affinity for the stationary phase of the compound during analysis (Lund 1994) and therefore correlation between the partition coefficient and the retention time was expected. The successful identification of such a correlation would enable possible prediction of the retention time of a compound for the experimental conditions, thereby reducing development time during future analytical method development. Correlation coefficients were calculated for both linear and logarithmic fit of each physicochemical property as a function of the retention time.

The resolution of the peaks of the internal standard and DMF was determined by the following equation:

$$R = 2(t_2 - t_1)/1.70(W_{1,h/2} + W_{2,h/2}) \quad (1)$$

Where t_1 and t_2 are the retention times of peaks 1 and 2 respectively and $W_{1,h/2}$ and $W_{2,h/2}$ are the peak width of peaks 1 and 2 respectively at half-height of the peak (USP 2006).

Table 3: Volumes of internal standard added to samples before analysis

Sample volume	Volume of internal standard added (µl)
100 ml	500
1 ml	5
200 µl	1
100 µl	0.5

3.3. Internal standard preparation

Internal standard was prepared by transferring 2.5 ml of 2-methoxyethanol into an amber glass bottle with a microliter pipette. Distilled water (7.5 ml) was then added to the content of the amber glass bottle in order to achieve a 25% concentration of the 2-methoxyethanol. The container was sealed and inverted each time directly before use and after preparation. The container was appropriately labeled and the material discarded after 30 days. The prepared internal standard solution was added to samples using a standard addition technique according to the volumes shown in Table 3.

3.4. Primary standard preparation

For the preparation of a primary standard during analysis, approximately 1 g of DMF was weighed accurately into a 100 ml A grade volumetric flask and the mass recorded. The flask was then made up to volume with distilled water and inverted twice to ensure proper mixing. The standard solution was then sampled by withdrawing 1 ml and transferring it into a clear glass injection vial (1.5 ml). This was done to mimic sample handling as closely as possible. The required volume of internal standard was then added to the standard (i.e. 5 µl). The sample vial was shaken to ensure a homogenous mixture before analysis.

3.5. Apparatus conditions

The system and conditions used for the GC analysis of DMF after optimization were as follows:

Apparatus:	Carlo Erba Strumentazione 4200 Gas Chromatograph
Detector:	Flame ionization detector (FID)
Column:	Glass Packed Column (2 m) with 10% Carbowax 20 m 80/100
Injection:	Manual injection, on column
Injection volume:	1 µl
Apparatus settings:	Input 10
Attenuation:	1
Injector temperature:	280 °C
Oven temperature:	180 °C
Detector temperature:	200 °C
Carrier gas pressure:	He: 2 kg/cm^2
Column gas pressure:	He: 1.5 kg/cm^2
Detector gas pressure:	H_2: 1.0 kg/cm^2 Air: 0.9 kg/cm^2
Instrument air flow rates:	He: 74.4 sccm (standard cubic centimeters per min) H_2: 39.3 sccm Air: 270.0 sccm

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