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Comparison Between Lc/Uv and Gc/Fid Techniques in Determining N,N-Dimethylacetamide (Dma) in Diacerein

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Abstract

Objectives: The aim of this work was the quantitative determination of N,N-Dimethylacetamide (DMA) as crystallization solvent in samples of Diacerein.

DMA is commonly used as a solvent in the chemical, agricultural and pharmaceutical industries. However, in order to ensure product quality and to protect patients from the potentially toxic properties, the substances used as active ingredients in therapeutic drugs should not contain high levels of residual solvents.

Methods: LC is commonly used in the pharmaceutical industry to check DMA in pharmaceutical products, but in this work we were interested in validating and comparing LC/UV and GC/FID techniques for determining the presence of DMA in Diacerein

Results: Both methods showed good linearity, precision and accuracy with comparable LOD and LOQ.

Conclusion: The GC method, however, since it uses DMSO as an internal standard, has higher analytical versatility, thus allowing the qualitative and quantitative determination of DMA at lower levels than those obtained with LC.

Keywords: N,N-Dimethylacetamide (DMA); Diacerein; GC/FID; LC/UV; Residual solvents

Introduction

N,N-Dimethylacetamide (DMA, CAS number 127-19-5) is a low-molecular-weight amide (87,12) with a high boiling point (165°C) and excellent solvent properties [1] and for this reason it is commonly used as a solvent in the chemical, agricultural and pharmaceutical industries.

European Pharmacopoeia Edition VIII defines residual solvents as "organic volatile chemicals that are used or produced in the manufacture of active substances or excipients, or in the preparation of medicinal products" [2].

Active substances as ingredients of therapeutic drugs should not contain high levels of residual solvents, in order to ensure product quality and to protect patients from potentially toxic properties. Diacerein, a drug with interleukin-1β inhibitory activity belonging to the class of DMARDs (Disease-Modifying Antirheumatic Drugs), is used to treat osteoarthritis and rheumatoid arthritis [3-14].

The aim of this work was quantitative determination of DMA in samples of Diacerein, synthetized from Aloin and purified using DMA as crystallization solvent (Figure 1).

LC and UV determinations are commonly carried in the pharmaceutical industry to check DMA in pharmaceutical products [15], in this work we were interested in a comparison between LC/UV and GC/FID [16] techniques for determining DMA.

Experimental

Materials and reagents

N,N-Dimethylacetamide and Pyridine were obtained from Fluka Chemie GmbH (Buchs, Switzerland); Ortho-phosphoric, Potassium phosphate monobasic acid 85%, Acetonitrile and Di-sodium hydrogen

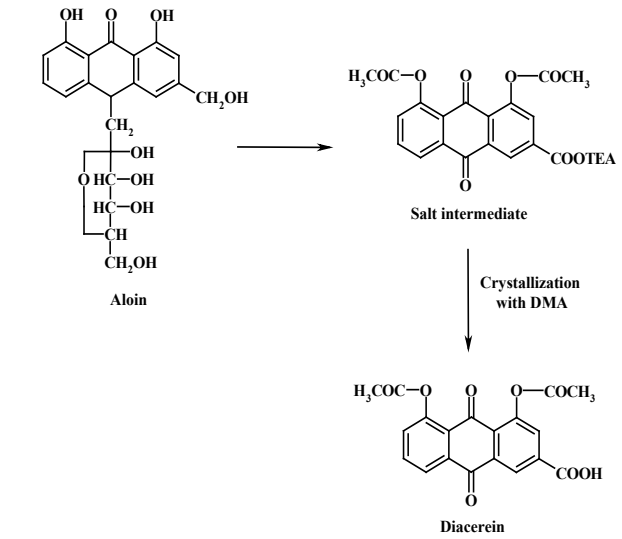

Figure 1: Synthesis of Diacerein.

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phosphate dihydrate from Merck (Darmstadt, Germany); NaOH and Methanol from J.T. Baker (Deventer, Holland); and Chloroform and Dimethyl sulphoxide (DMSO) from RdH (Seelze, Germany).

Water was obtained from Milli-Q ultra-purifying system, 18.2 MΩ/cm (Millipore SA-67120, Molscheim, France). All solvents and reagents used were of analytical grade.

Diacerein was provided by Laboratoires Medidom S.A. (Geneva, Switzerland).

Apparatus

GC analysis was performed on a Trace GC (ThermoQuest) gas chromatograph with an

AS 2000 automatic sampler and FID detector.

LC analysis was performed on a Hewlett-Packard (Palo Alto, USA) model 1050 chromatograph with autosampler and UV detector.

GC conditions: The column used was a Megasolve capillary column (50 m x 0.32 mm i.d., 0.25 µm, Mega, Legnano, Italy).

Purified helium was used as carrier gas at constant pressure to ensure a flow rate of 1 mL/min. Detector gas flow rates were 9 mL/min and 350 mL/min for hydrogen and air respectively. Column temperature was constant at 120°C. Injector temperature was 200°C and detector temperature 250°C. One µL of sample was injected at a split rate of 15:1.

In these chromatographic conditions, DMA and IS retention times (t_R) were about 5.8 and 11.3 minutes respectively.

LC conditions: The column used was a reversed phase column LiChroCART® 250-4 (Lichrosphere 100 RP18, 125 x 4 mm i.d.-5 µm, Merck, Darmstadt, Germany) with a LiChroCART® precolumn (Lichrosphere 100 RP18, 125 x 4 mm i.d.-5 µm, Merck).

Injection was effected through a 20 µL loop. Analysis was performed using mobile phase A, a mixture of phosphate buffer (pH=5.5) - methanol (98:2, v/v), and mobile phase B, a mixture of phase A - acetonitrile (50:50, v/v), at the gradient shown in Table 1.

Flow rate was 1 mL/min; the column was maintained at room temperature and detection was effected at 220 nm.

In these conditions, DMA retention time was about 11.4 minutes.

Standard solutions

DMA standard solution for GC analysis: 5 µL of DMA were placed in a 10 mL volumetric flask, with pyridine as diluent (500 nL/mL). 2 mL of DMA solution were transferred into another volumetric flask and added with 8 mL of pyridine (100 nL/mL).

IS solution for GC analysis (IS): 20 µL of DMSO were dissolved in 10 mL of chloroform.

DMA standard solution for LC analysis: 100 µL of DMA were placed in a 100 mL volumetric flask and dissolved in water (1000 nL/mL). 10 mL of the resulting solution were diluted to 100 mL with the same solvent (100 nL/mL).

Working standard samples for GC analysis

Two working standard samples (CR1 and CR2) were prepared, CR1 by diluting 1 mL of DMA standard solution with 9 mL of pyridine, and CR2 by diluting 0.2 mL of DMA standard solution with 9.8 mL of pyridine. 1.5 mL of both solutions were placed in a tube and added with 20 µL of IS. The resulting solutions were warmed to 80°C and cooled in ice. An aliquot of each filtered solution was transferred to a vial for analysis.

The final concentration of CR1 was 10 nL/mL, corresponding to 14.4 µg total, and that of CR2 was 2 nL/mL, or 2.9 µg total.

Working standard samples for LC analysis

5 mL of NaOH 1 N, 1 mL of DMA standard solution and 1 mL of ortho-phosphoric acid 20% were placed in a 20 mL volumetric flask. Water was used as diluent.

Sample preparation for GC analysis

Exactly weighed quantities of 100 mg of Diacerein, 1.5 mL of pyridine and 20 µL of IS were placed in a tube. Each sample was warmed to 80°C until a yellow solution was obtained, which was cooled in ice to precipitate the Diacerein. This was then eliminated by filtration and an aliquot of solution was transferred to a vial for analysis.

Sample preparation for LC analysis

An exactly weighed quantity of 1 g of Diacerein was dissolved in 5 mL of NaOH 1N, the resulting solution was added with 14 mL of water and the Diacerein was precipitated with 1 mL of ortho-phosphoric acid 20%. This was then eliminated by filtration before analysis.

Results

Diacerein was analyzed by LC and GC to verify the presence of N,N-dimethylacetamide (DMA, crystallization solvent). Both methods proved the absence of DMA in Diacerein samples (Figures 2-4).

The LC and GC methods were validated, showing good linearity, precision and accuracy, with comparable LOD and LOQ (Table 2).

Specificity

No interferences at the retention time of the substances (DMA or IS) were found in analyses of placebo and placebo spiked with DMA and IS (Figure 1).

Time (min)	Phase A (%)	Phase B (%)
0	100	0
20	100	0
26	0	100
40	0	100
46	100	0
50	100	0

Table 1: Elution gradient for LC analysis.

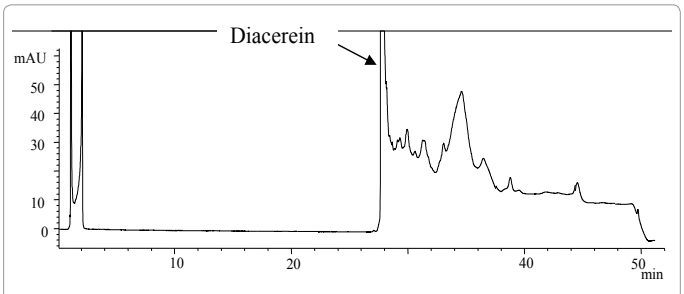

Figure 2: LC chromatogram of Diacerein working standard.

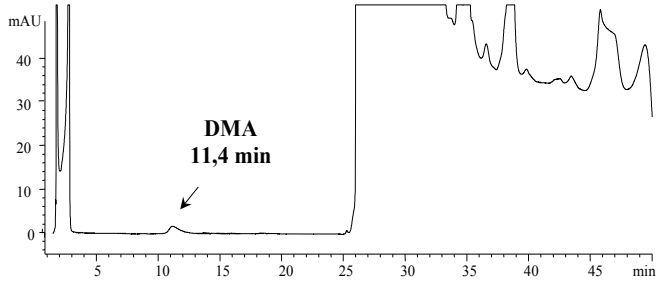

Figure 3: LC chromatogram of Diacerein working standard spiked with DMA standard solution (25 ppm).

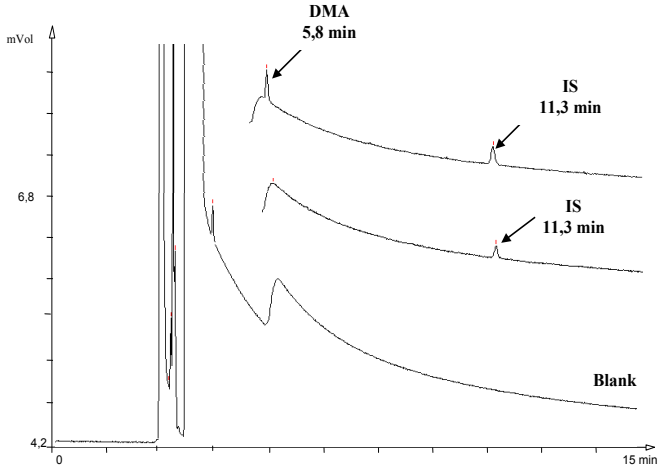

Figure 4: GC chromatograms of a) Blank , b) Blank spiked with IS, c) Blank spiked with DMA standard solution.

Linearity

The linearity of the LC and GC methods was evaluated on three sets of five standard samples in the range 1.25-100 nL/mL, preparing calibration curves 1, 2 and 3 for each set and a total curve on all 15 samples (calibration curve C). Linear regression lines were obtained by the least squares method, plotting peak areas (A_{DMA} for LC, ratios of DMA peak area to IS peak area for GC) versus DMA concentration (C_{DMA}) in each standard sample (Tables 2 and 3).

The remaining tables show the equations (y = ax + b) of calibration curves 1, 2, 3 and C, and the corresponding correlation coefficients (R^2) for LC and GC.

Response ratio (RR)

The Response Ratio (RR) is defined as the relationship between the peak area (A_{DMA} for LC, ratios of DMA peak area to IS peak area for GC) and analyte concentration:

$$RR = \frac{A}{C_{DMA}}$$

where:

A = DMA peak area for LC; ratios of DMA peak area to IS peak area for GC

A_{IS} = IS peak area

C_{DMA} = DMA concentration (µg total) in standard samples.

The mean Response Ratio (RR_{mean}) is the mean of all RRs calculated in the linearity study.

Precision and accuracy

Intra-day and inter-day precision values were evaluated by the RR_{mean} and the coefficient of variation (CV%) from linearity data. As regard as accuracy six replicates were carried out as in the case of intra-day precision. For inter-day precision 3 concentrations were analyzed in triplicate for three consecutive days.

Accuracy was calculated as the recovery percentage between found and known concentrations (Rec% = experimental value/theoretical value x 100).

The results showed the high degree of precision and accuracy of both analytical methods.

All statistical parameters of precision, accuracy, LOQ and LOD are listed in Table 4.

Sensitivity

LOD and LOQ were evaluated progressively diluting standards solutions of the active principles. LOD was determined as the lowest detectable analyte concentration which was at least 3 times higher than the standard deviation of the signal to noise ratio. LOQ was determined as the lowest analyte concentration which could be quantified and was at least 10 times higher than the standard deviation of the signal to noise ratio.

	a	b	R^2
Calibration curve 1	12.512	-0.8041	0.9999
Calibration curve 2	12.695	-2.4975	0.9999
Calibration curve 3	12.652	1.7518	0.9999
Calibration curve C	12.617	-0.4419	0.9998

Table 2: Statistical linearity parameters of LC method.

	a	b	R^2
Calibration curve 1	0.0296	0.0372	0.9995
Calibration curve 2	0.0304	0.0215	0.9995
Calibration curve 3	0.0294	0.0473	0.9993
Calibration curve C	0.0298	0.0354	0.9992

Table 3: Statistical linearity parameters of GC method.

	LC/UV	GC/FID
INTRA-DAY PRECISION		
RR mean	12.481 – 12.783	0.046 – 0.047
CV%	1.9 – 2.6	4.1 – 8.2
INTER-DAY PRECISION		
RR mean	12.590	0.031
CV%	2.4	6.6
SD	0.296	0.002
ACCURACY		
% Recovery	99.7-100.5	94.4-105.5
LOQ (ppm)	25	17
LOD (ppm)	12.5	7

Table 4: Statistical parameters of precision, accuracy, LOQ and LOD.

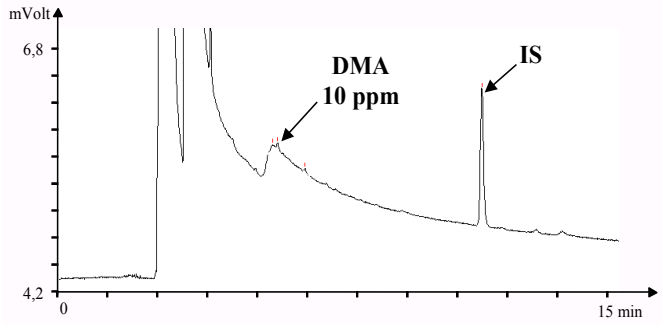

Figure 5: GC chromatogram of concentrated sample of Diacerein.

Discussion

Two different analytical methods for the determination of diacerein were optimized and compared. As regard as specificity, in both methods the interference of DMF and DMSO, solvents with characteristics similar to DMA, were taken into account. None of the two solvent showed peaks interfering with DMA. The HPLC method was provided to us by the company interested in the determination of DMA as an impurity in Diacerein synthesis. We optimized this method lowering the LOD and LOQ. As it is evident in Figure 2, after 50 min the chromatogram rapidly reaches baseline. Nevertheless we allowed the column to equilibrate for ten minutes after each analysis. This method did not provide for the use of an internal standard. We did not study a molecule suitable for this use, because we were interested in developing a GC method, which resulted more adequate in terms of sensitivity. On the other hand the two method were comparable in terms of accuracy and precision.

Conclusions

N,N-Dimetilacetammide (DMA) was quantitatively analyzed in samples of Diacerein, using both LC/UV and GC/FID. All samples turned out to be lacking in DMA within the sensitivity limits of the two methods.

Although both methods were shown to be sufficiently sensitive, GC/FID has higher analytical versatility than LC, since it employs an internal standard (DMSO). It was precisely this internal standard which allowed us to increase the sensitivity of the method by partially evaporating the samples, prepared as described previously, and very low levels of DMA (about 3 ppm) (Figure 5) were found.

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