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Note

Determination of residual dimethyl sulphate in a lipophilic bulk drug by wide-bore capillary gas chromatography

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Dimethyl sulphate is widely used as a methylating agent for organic synthesis. It is volatile and readily hydrolysable so it seems unlikely that dimethyl sulphate would remain in any bulk drug substance after final processing. However, to verify that this potentially hazardous material[1] is not present, a routinely applicable gas chromatographic–flame ionization detection (GC–FID) method capable of detecting low levels was developed.

Previously published methods[2,3] both made use of packed-column GC with consequent high limits of detection and long analysis times. The method described below makes use of a J&W DB-1 Megabore, (30 m × 0.53 mm I.D., film thickness 5 μm) fused-silica wall-coated open-tubular (WCOT) wide-bore capillary column. This column was chosen because it is stable, inert and robust and is widely available. Normally, material like dimethyl sulphate would be chromatographed using a more polar stationary phase, but the inertness of the fused-silica column maintains good peak shape and the relatively thick film prevents column overload.

Hexane was chosen as the solvent because the drug is highly soluble in this solvent, it is inert to attack by dimethyl sulphate and its volatility is not excessive. The poor response of the flame ionization detector to dimethyl sulphate, due to the low carbon content and high oxidation state of the molecule, forced the use of a high concentration of drug in samples (approximately 10%, w/w) in order to achieve a sufficiently low limit of detection. Greater sensitivity may well be achievable using, say, a sulphur-specific detector although such detection systems may not be routinely available to all laboratories.

Matrix effects such as interfering peaks from the drug or trapping of the dimethyl sulphate by drug residues in the injector port were shown to be absent.

Loss of dimethyl sulphate from spiked samples of bulk drug was also investigated and losses (probably by volatilisation) were found to be marked when spiked drug was exposed to open atmosphere.

The method is linear, rapid (analysis time less than 5 min) and precise. It is suitable for use as a routine quantitative assay for dimethyl sulphate and is likely to be applicable to a variety of other bulk drug materials.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a Hewlett-Packard 5890 gas chromatograph with a Hewlett-Packard 7673A auto-injector, a Hewlett-Packard split/splitless injector, a J&W DB-1 Megabore 30 m × 0.53 mm I.D. column, (5 μm film thickness), a Hewlett-Packard flame ionization detector and a Hewlett-Packard 3350A laboratory automation system for signal integration.

Conditions

Carrier gas: helium at 51 kPa (7.5 p.s.i.) producing a linear gas velocity of 59 cm s^{-1}. Injector: 140°C, splitless mode, purge after 30 s. Detector: 200°C, hydrogen at 150 kPa (22 p.s.i.), air at 250 kPa (37 p.s.i.). Injection volume: 2 μl. Oven temperature: 100°C. Split vent flow: 40 ml min^{-1}. Septum purge: 4 ml min^{-1}. Amplifier range: 2^5.

Materials

Hexane solvent (HPLC-grade) was obtained from FSA (Loughborough, U.K.). The dimethyl sulphate (99 + % Gold Label) was obtained from Aldrich (Gillingham, U.K.).

Preparation of standards

Standards were prepared in hexane at concentrations of 3–700 μg ml^{-1} dimethyl sulphate (nominally) with 5 nl ml^{-1} toluene as internal standard. Weighing of the dimethyl sulphate was carried out in a sealed syringe with the dimethyl sulphate being dispensed directly into the solvent after the weighing operation. This was done to prevent any loss by evaporation.

Preparation of samples

Samples were prepared by accurately weighing approximately 100 mg of the bulk drug into a suitable vial and adding 1.00 ml of an internal standard solution containing 5 nl ml^{-1} toluene in hexane. The vial was then sealed and the drug dissolved with gentle warming. A 2-μl volume of this solution was injected into the GC system using the conditions described above. All manipulations were carried out quickly and containers were kept closed with minimum headspace whenever possible in order to minimise evaporation as well as to reduce operator exposure to dimethyl sulphate.

RESULTS AND DISCUSSION

Linearity, precision and method performance

A typical chromatogram for a 100-μg ml^{-1} standard of dimethyl sulphate with toluene as internal standard is shown in Fig. 1. Linearity of response was established for solutions containing 3–700 μg ml^{-1} dimethyl sulphate with toluene as internal standard. The following regression line was calculated for the data:

$$\text{Response ratio} = 0.01027\,[(CH_3)_2SO_4]\ (\mu g\ ml^{-1}) - 0.01003$$

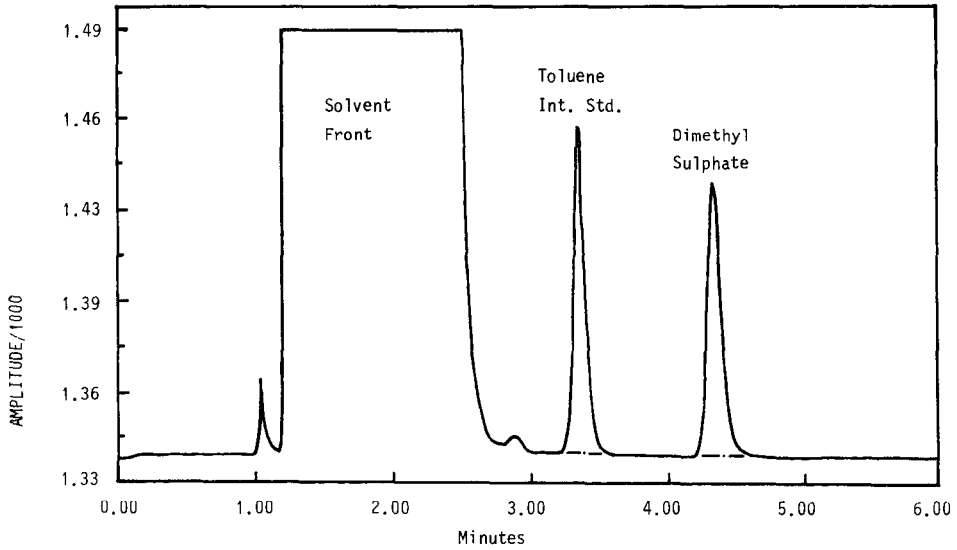

Fig. 1. Typical chromatogram for a 100-μg ml^{-1} dimethyl sulphate standard.

with a correlation coefficient (r) of 1.000. The probability that intercept is zero is 0.0497. The small negative intercept was probably due to the difficulty the integration system had in assigning an accurate baseline to the dimethyl sulphate peak in the low-concentration standards. This in turn was due to the digital noise seen in the signal recorded by the integrator (see Fig. 2 and also *Limit of detection* for further

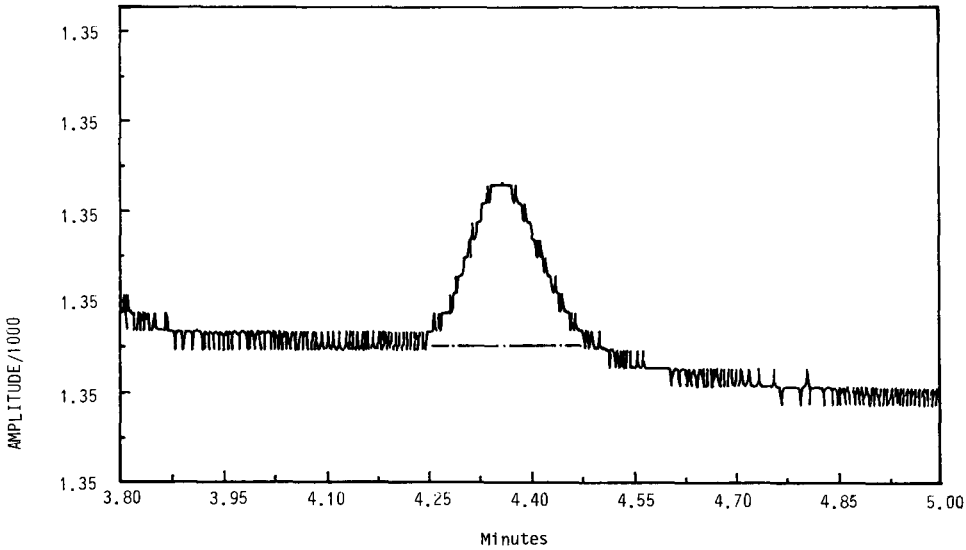

Fig. 2. Typical dimethyl sulphate peak for a 3-μg ml^{-1} standard. Note high baseline assignment.

discussion). The negative intercept would only be significant at low concentrations so quantitation is best carried out using at least bracketing standards when low levels are being determined.

The response ratios from six replicate injections of a 100 μg ml^{-1} standard containing 5 nl ml^{-1} toluene as internal standard gave a relative standard deviation (R.S.D.) of 0.52%, showing that the method exhibits good precision. Peak shape was found to be good, even at 700 μg ml^{-1}. However, chromatographic efficiency was quite poor for this type of system with only 4884 effective plates being generated for the 700 μg ml^{-1} dimethyl sulphate peak. This phenomenon may well be due, at least in part, to the use of a large volume (2 μl) injection in the splitless mode; a retention gap may help to alleviate this. It may also be that the mass-transfer kinetics are particularly slow for this material.

No interfering peaks were observed in the batch of hexane used. Furthermore, trapping of dimethyl sulphate by the bulk drug injected with the samples was shown to be absent: approximately 100 mg drug were added to 1.00 ml of the 70 μg ml^{-1} standard and the assay results obtained with and without added drug were not significantly different.

On account of the large amount of drug substance being injected, it is necessary to "bake out" the GC column every so often by raising the injector and detector temperatures to about 250°C and the column temperature to about 225°C for at least 1 h. It is also advisable to inject clean solvent to wash the injector port.

It was noted that using such a high concentration of bulk drug in the sample preparation could lead to the drug recrystallising from the sample solution after a few hours. This is unlikely to present a problem since the analysis time is so short and furthermore, the recrystallisation of the bulk drug is unlikely to affect the much lower concentration of dimethyl sulphate in the supernatant.

Limit of detection

The limit of detection was estimated by calculating the concentration of dimethyl sulphate at a signal-to-noise ratio of 3. This was done using a 3 μg ml^{-1} standard of dimethyl sulphate. The chromatogram is shown in Fig. 2 and from this, the limit of detection was estimated to be 1.2 μg ml^{-1} dimethyl sulphate which is equivalent to 12 μg g^{-1} dimethyl sulphate in bulk drug.

The limit of detection may be improved slightly by decreasing the "Range" setting on the HP 5890 amplifier but excessive amplification results in a noisy baseline and the gain in sensitivity achieved by this approach is limited.

Loss of dimethyl sulphate from bulk drug after spiking

Spiking of the bulk drug with a known level of dimethyl sulphate proved to be awkward on account of the ease with which dimethyl sulphate is lost on contact with open atmopshere. A primary objective in such an experiment is to ensure a homogeneous distribution of the spike throughout the bulk drug; the first attempt at achieving this centred around dissolving the drug in a solution of dimethyl sulphate in hexane and evaporating the hexane over a stream of nitrogen. Unfortunately, virtually all of the dimethyl sulphate was lost, probably by evaporation, using this approach.

The next approach taken was to melt the drug (1.9287 g) and add a known amount of dimethyl sulphate (9.8 mg) from a syringe directly to the melt in a stop-

TABLE I

LOSS OF DIMETHYL SULPHATE FROM SPIKED BULK DRUG STORED IN A CLOSED CONTAINER

Sampling time (h)	*Concentration dimethyl sulphate in bulk drug (%, w/w)*	*Percentage of initial concentration*
0	0.415	100.0
2	0.359	84.7
4	0.340	80.3
45	0.298	70.4
48	0.266	62.8

pered flask. The contents were then swirled vigorously. On cooling the spiked drug solidified and was then broken up using a spatula to give smaller pieces. This method was quite successful giving a recovery of 83.6% of the initial spiking level when analysed using the above method. This spiking process therefore resulted in a sample of bulk drug containing 0.424% (w/w) dimethyl sulphate. Good agreement between replicate analyses implied that the spiking was homogeneous.

Duplicate samples of the spiked drug were taken and assayed for dimethyl sulphate at intervals of 2, 4, 45 and 48 h. Between sampling, the spiked drug was kept in a tightly stoppered 50-ml conical flask. The results of the assays are shown in Table I. Approximately 4–10% of the dimethyl sulphate content was lost at each sampling regardless of the time between samples. This implies that the loss of the dimethyl sulphate is not due to degradation by reaction with the bulk drug and is probably caused by volatilisation on sampling. However degradation by, for example, moisture in the headspace has not been ruled out.

This loss was further investigated by placing the conical flask containing the spiked drug in a fume cupboard with the fan on and the flask stopper removed. Initial duplicate samples were taken and assayed as before followed by subsequent duplicate samples at 1, 2.5 and 5 h. The results, given in Table II, show that loss of the dimethyl sulphate is quite rapid despite the bulk drug existing as coarse aggregates. This sup-

TABLE II

LOSS OF DIMETHYL SULPHATE FROM SPIKED BULK DRUG STORED IN AN OPEN CONTAINER

Sampling time (h)	*Percentage of initial concentration*
0	100.0
1	56.6
2.5	48.7
5	15.5

ports the view that the dimethyl sulphate is lost on account of its exposure to the atmosphere and not because of any reaction with the bulk drug.

CONCLUSIONS

Dimethyl sulphate was chemically stable in the bulk drug but exposure to an open atmosphere results in significant loss of dimethyl sulphate. Sampling should, therefore, be carried out in such a way as to minimise exposure of the bulk drug to the atmosphere. On examination, none of the batches of bulk drug manufactured to date was found to contain any dimethyl sulphate at or above the limit of detection of 12 $\mu g\ g^{-1}$.

REFERENCES

1 M. Windholz (Editor), *The Merck Index*, Merck & Co., Rahway, NJ, 9th ed., 1976, p. 433, No. 3246.
2 N. M. Turkevich, S. I. Tereshchuk and O. M. Tereshchuk, *Farmatsiya (Moscow)*, 27 (1978) 79–80.
3 I. A. Zheltukhin, N. I. Glybochko, A. S. Sobolev and T. S. Maslakova, *Zavod. Lab.*, 50 (1984) 21.