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# Determination of N, N-Dimethylaniline in Penicillins by GC-MS

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**Abstract**□ A quantitative GC-MS spectrometric assay was used for the determination of residual N,N-dimethylaniline as a contaminant in commercial penicillin derivatives from various sources. The assay utilizes selective ion focusing to monitor in a GC effluent the molecular ions of DMA generated by electron impact ionization. This method includes dissolution of the sample in alkaline solution, extraction of organic base with cyclohexane and injection into GC-MS with a 3% OV-17 column. Levels of 50 ppb of DMA were easily measured with a coeffecient of variation less than 5% and recoveries from spiked samples exceeded 97%. The results of the determinations of DMA in various commercial penicillins were relatively free of this contaminant.

**Keywords**□N,N-Dimethylaniline-GC-MS analysis in penicillins; penicillins-N,N-Dimethylaniline.

The premarketing certification process assures that each bulk and dosage form of antibiotics intended for human use complies with the specifications of proposed and established standards for identity, potency, quality, and purity.

The Code of Federal Regulations of America provides for Good Manufacturing Practices (GMP) in the production of pharmaceuticals. The direction to "...minimize contamination of products by extraneous adulterants..." applies also to residual reagents that may exhibit undesirable properties such as toxicity or carcinogenicity during antibiotic theraphy and/or possible accumulation from other drugs.

Ampicillin, a semisynthetic penicillin, has been synthesized through diverse routes[1] by using 6-aminopenicillanic acid as an intermediate and an organic base such as N,N-dimethylaniline (DMA) to abstract generated hydrogen chloride, the presence of HCl inhibits the synthesis.

But DMA remains occasionally in the final products because of high boiling point (192-4°). The pharmaceutical properties of DMA have not been fully elucidated. But it seemed advisable to limit its presence because of dubious nature and possible carcinogenicity.

For the qualitative determinations of DMA paper chromatography[2~3], thin layer chromatography[4], gas chromatography[5] and nitration method[6] have been known and nowadays gas chromatography[7~8], thin layer chromatography and densitometry[9]

have been attempted for the quantitative analysis.

This paper describes a GC–Mass spectrometric assay of DMA in commercial samples. Selective ion monitoring, the technique built on combined GC–Mass spectrometry with selective focusing on suitable fragments of molecular ion (mass fragmentography) or the molecular ion itself, is a well-established technique used widely in pharmacology[10~11]. This technique was used to develope a senstive and specific assay for DMA in bulk and dosage form.

It is possible to detect the m/e 121 molecular ion of DMA at the level of 50ppb in cyclohexane.

The DMA of ampicillin trihydrate (Amp·$3H_2O$) ampicillin sodium (Amp·Na) and cloxacillin were analized by this method.

## EXPERIMENTAL

*Materials*

Analytical grade N,N–dimethylaniline was purchased from Hayashi Co., which was used without further purification. Cyclohexane was purchased from E. Merk, Darmstadt. It was purified by a general method. Commercial Amp·$3H_2O$, Amp·Na and cloxacillin were used. The other reagents used were analytical grade.

*Apparatus*

An AEI 1073 gas chromatograph–mass spectrometer was equipped with a 1.5m×4mm I.D. glass column, packed with 80–100 mesh Gas ChromeQ loaded with 3% of OV–17. The flow rate of carrier gas(helium) was ca. 30ml/min. The gas chromatograph was connected with the mass spectrometer through all glass separator.

A vacuum diverter system was used to prevent the solvent from contaminating the source of the mass spectrometer which was used in electron impact mode with the multiple ion detection unit tuned at m/e 121.

The column was conditioned for 24hr. at 250°C. The column temperature, injection block, molecular separator and ion source were maintained at 150, 250, 200 and 200°C respectively. The chromatograms were re corded with the total ion current monitor (TICM). Mass fragmentograms were taken at 70eV with an accelerator voltage of 1Kv and the signals were measured on a SE oscillograph 3006 UV recorder with a chart speed of 50mm/min.

*Standard Solution*

N,N–dimethylaniline was weighed about 25mg accurately in a 50ml volumetric flask and added 1ml of concentrated hydrochloric acid and about 20ml of water. It was shaked to dissolve on a mixer and diluted to volume. This sloution was used to make standard solutions of varying concentrations ranging from 0.5 to 5μg/ml.

*Sample Preparation*

Amp·$3H_2O$ , Amp·Na and Clox. were weighed about 1g accurately in a 10ml screw cap culture tube. 2ml of 1.25M NaOH was added and shaked until dissolved.

*Procedure*

1ml cyclohexane was added and shaked vigorously for about 1min. and allowed the phases to separate. From the upper cyclohexane phase 2μl sample was drawed and

injected into the GC-MS and recorded the fragmentogram of m/e 121.

*Calculations*

The height of each peak is measured

Then: $X=\frac{FHt}{FHs} \times 2\,(\mu g/ml) \times \frac{V(ml)}{W(g)}$

where X is the concentration of DMA(ppm), FHs and FHt are peak heights of standard and sample fragmentogram respectively, V is the volume of sample(ml) and W is the weight of sample (g).

## RESULT AND DISCUSSION

*Choice of Liquid Phase*

DMA is relatively nonploar because of the alkyl shielding. For this reason two kinds of liquid phase were compared. One is polar liquid phase PEG 20M (carbowax 20M), and another is intermediately polar liquid phase, OV-17.

In the case of PEG 20M the chromatogram (Fig. 1) of DMA show a tailing a little and Mass fragmentogram (Fig. 3) show a tailing significantly.

But in the event of OV-17 the chromatogram (Fig. 2) and mass fragmentogram (Fig. 4) gave the best resolution and the best concentration of stationary phase was found to be 3%

*The Temperature of the Column*

For the purpose of obtaining suitable column temperature, DMA standard solutions (5$\mu$g/ml) were injected at 90, 120, 150, 180, 210°C of column temperature with 3% OV-17 column.

As a result the fragmentograms had tailings and long retention times until 120°C but the fragmentogram were influenced by bleeding of packing materials above 180°C. From above results 150°C was selected as a column temperature and the retention time of DMA was 3min.

According to the temperature, retention time and peak shape were appeared as Table I.

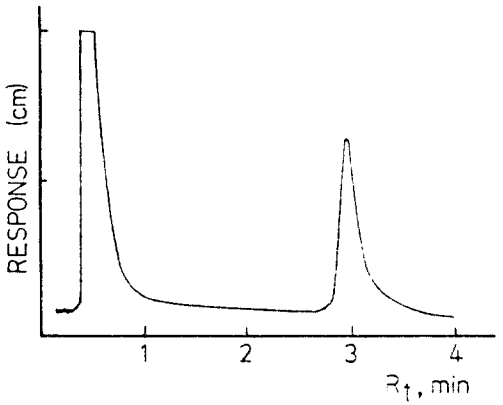


**Fig. 1:** Chromatogram of DMA.
1.5m×4mm glass column 10% PEG 20M on Shim alite W. 80/100mesh at 180°C, He ca. 30ml/min. Standard sol'n: 5ppm, 2$\mu l$.

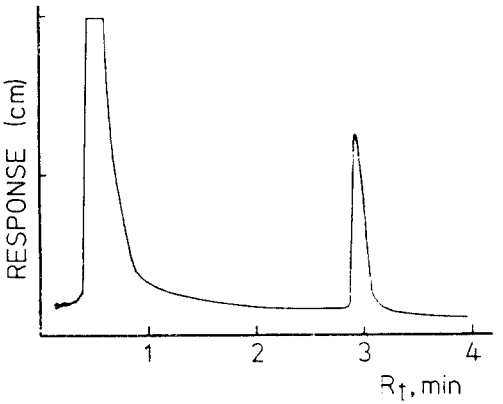


**Fig. 3:** Chromatogram of DMA.
1.5m×4mm glass column 3% OV-17 on Gas chrome Q, 80/100mesh at 150°C, He ca. 30ml/min. Standard sol'n: 5ppm, 2$\mu l$.

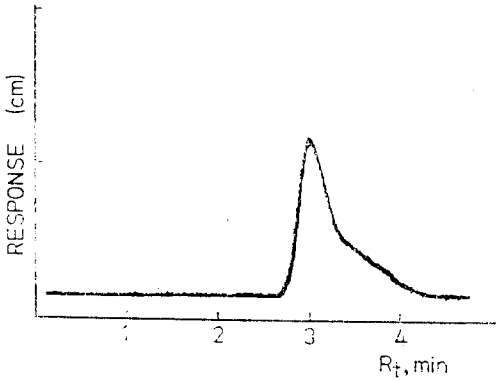


**Fig. 2:** m/e 121 Fragmentogram of DMA with 10% PEG 20M.

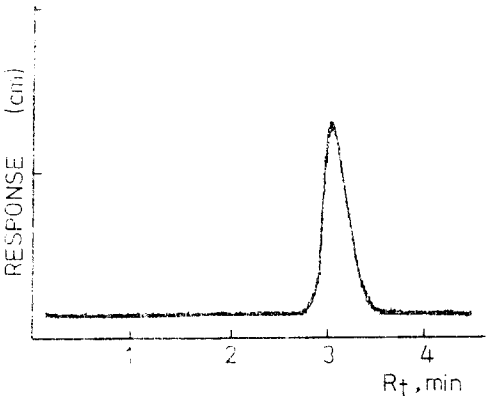


**Fig. 4:** m/e 121 Fragmentogram of DMA with 3% OV-17.

**Table I: Comparision of column temperature.**

| Temp. (°C) | Retention Time | Remark |
|---|---|---|
| 90 | 7min, 15sec. | tailing |
| 120 | 5min, 10sec. | tailing |
| 150 | 3min. | sharp, no bleeding |
| 180 | 2min, 15sec. | bleeding |
| 210 | 1min, 45sec. | bleeding |

*Mass Spectrum of DMA*

DMA has a strongly electron donating substituent such as dimethylamino group. Dimethylamino group can accomodate two positive charges within the benzene ring and the molecular ion is stable enough to be detected. The molecular ion peak is very intensive and fragmention peaks are obesarved a little (Fig. 5). The interpretation of the mass spectrum was shown at Table II.

*Calibration*

Standard solutions containing from 0.5 to 5$\mu$g/ml of DMA drawed a 2$\mu$l carfully and injected it into GC-MS.

Fig. 6 shows mass fragmentograms of standard sol'ns 1,2,3ppm of DMA and of

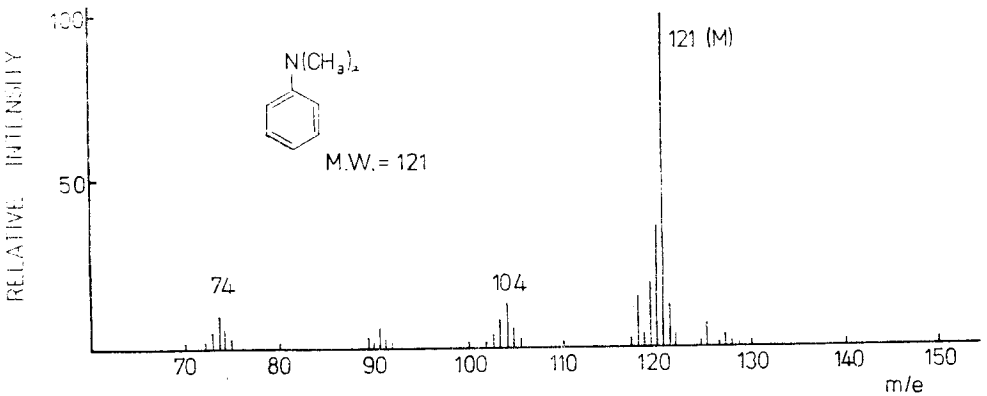


**Fig. 5:** Mass spectrum of DMA.
GC-MS: AEI MS 1073 & pye Unicam 104 GC.
Column : 3% OV-17 on Gaschrome Q 80/100mesh 1.5m×4mm I.D. 150°C isothermal.
Carrier gas : He 30ml/min. GC-MS interoven: 200°C
Electron energy: 70eV Sample size : 2$\mu$l

**Table II: Formal interpretation and intensity of the mass spectrum of DMA.**

| m/e | Relative intensity | Ion fragment |
|---|---|---|
| 121 | 100 | $M^+$ |
| 120 | 34 | $M-H^+$ |
| 74 | 3 | $H-C^+(=C=)_4\ C^+-H$ |
| 119 | 11 | $M-H_2^+$ |
| 117 | 10 | $M-2H_2^+$ |
| 122 | 8 | $M+H^+$ |
| 104 | 7 | $M-CH_5^+$ |

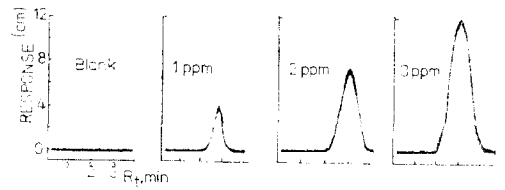


**Fig. 6:** Mass fragmentogram at m/e 121 of cyclohexane and standard sol'ns.

blank cyclohexane.

Fig. 7 is a calibration graph for these low contents of DMA in cyclohexane obtained using standards sol'ns with concentrations ranging from 0 to 5ppm of DMA.

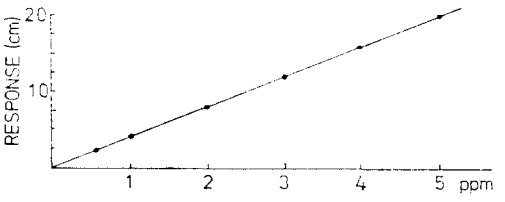


**Fig. 7:** Calibration graph for cyclohexane sol'n containing 0.5, 1,2,3,4,5ppm of DMA.

*Reproducibility*

Method reliability was verified by spiking samples of ostensibly DMA-free ampicillin with different amount of standard.

**Table III: Determination of DMA in standard mixtures by GC-MS.**

| Added ($\mu$g) | Found ($\mu$g) | Recovery (%) |
|---|---|---|
| 0.5 | 0.486 | 97.20 |
| 1 | 0.987 | 98.70 |
| 2 | 2.101 | 105.05 |
| 3 | 2.993 | 99.77 |
| 4 | 4.165 | 104.13 |
| 5 | 5.203 | 104.06 |
| 6 | 5.898 | 98.30 |
| 7 | 7.325 | 104.64 |
| M.V.=mean value | | M.V.=101.48% |
| C.V.=coeffecient of variation | | C.V.=3.03% |

Recoveries in excess of 97% were obtained. And the coefficient of variation was 3.03% (n=8). Each recoveries were as follows (Table III).

*Application to the commercial samples*

Results of the determination of DMA in various commercial penicillins are summarized in Table IV.

**Table IV: Dimethylanilline in various penicillins.**

| Sample | | Type | ppm |
|---|---|---|---|
| Amp·$3H_2O$ | A | bulk | 0.91 |
| | B | bulk | 12.47 |
| | C | bulk | 5.45 |
| | D | bulk | 7.65 |
| | E | bulk | 9.37 |
| | F | bulk | 2.74 |
| | G | capsule | 6.32 |
| Amp·Na | A | bulk | 1.02 |
| | B | bulk | 2.21 |
| Clox. | A | bulk | 0.72 |
| | B | bulk | 3.07 |

## CONCLUSION

The determination of the molecular ion

m/e 121 of DMA in cyclohexane was possible at the level of 50ppb.

Results of the determination of DMA in various commercial penicillins were relatively free of this contaminant. Occasionally the amount of residual DMA was above 10ppm but it does not become an issue accoring to foreign regulations.

GC-MS spectrometry is expected to apply residual reagents of the other drugs.

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