NITROFURAZONE

Method no.	PV2069
Matrix:	Air
Target Concentration:	0.1 mg/m³
Procedure:	Samples are collected by drawing known volumes of air through glass fiber filters. The filters are extracted with dimethylformamide (DMF) and analyzed by high performance liquid chromatography (HPLC) using an ultraviolet detector (UV).
Recommended air volume and sampling rate:	240 L at 1.0 L/min
Detection limit of the overall procedure (based on the recommended air volume and the analytical detection limit):	0.005 mg/m³
Special precautions:	Protect the cassette from being exposed to light at all times by wrapping it with foil.
Status of method:	Stopgap method. This method has been partially evaluated and is presented for information and trial use only.

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1. General Discussion

1.1 Background

1.1.1 History of procedure

This evaluation was undertaken because OSHA recently received samples requesting the analysis of nitrofurazone. The samples had been collected on glass fiber filters. This describes the method developed for the sampling and analysis of nitrofurazone.

1.1.2` Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

Oral toxicity tests have been done on rats. The results of these tests showed an increased incidence of benign mammary tumors, which was insufficient to evaluate the carcinogenicity of nitrofurazone. (Ref. 5.1) The oral LD_{50}'s for mice and rats are 249 mg/kg and 590 mg/kg respectively. (Ref. 5.3)

1.1.3 Potential workplace exposure

Nitrofurazone is used as an antibacterial agent in human medicines and veterinary medicine. (Ref. 5.1) There was no information available on the number of workers exposed to nitrofurazone each year.

1.1.4 Physical properties (Ref. 5.1 to 5.3)

CAS number:	59-87-0
IMIS number:	N905
Molecular weight:	198.16
Molecular formula:	$C_6H_6N_4O_4$
Melting point:	236-240˚C decomposes
Solubility:	Soluble in dimethylformamide, polyethylene glycol; slightly soluble in propylene glycol, acetone; very slightly soluble in water; almost insoluble in chloroform, benzene
Chemical name:	5-nitro-2-furaldehyde semicarbazone
Synonyms:	Aldomycin; Alfucin; Amifur; Babrocid; Becafurazone; Biofuracina; Biofurea; Chemofuran; Chixin; Cocafurin; Coxistat; Dermofural; Dynazone; Eldezol F-6; Fedacin; Flavazone; Fracine; Furacilin; Furacillin; Furacin; Furacin-E; Furacine; Furacinetten; Furacine-HC; Furacoccid; Furacort; Furacycline; Furaldon; Furalone; Furametral; Furan-ofteno; Furaplast; Furaseptyl; Furaskin; Furaziline; Furazin; Furazina; Furazol W; Furazone; Furesol; Furfurin; Furosem; Fuvacillin; Hemofuran; Ibiofural; Mammex; Mastofuran; Monofurazan; NSC-2100; Otofural; Otofuran; Rivafurazon; Rivopon-S; Sanfuran; Spray-Dermis; Sprayforal; Vabrocid; Vadrocid; Yatrocin
Description:	A microcrystalline, lemon-yellow, odorless solid; darkens on prolonged exposure to light

UV Scan:

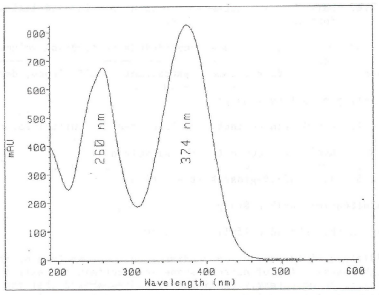
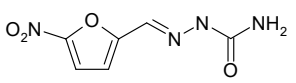


Figure 1.
UV Scan of Nitrofurazone in Mobile Phase

Structure:



1.2 Limit defining parameters

The detection limit of the analytical procedure is 1.25 ng per injection. This is the amount of analyte which will give a peak whose height is approximately five times the baseline noise.

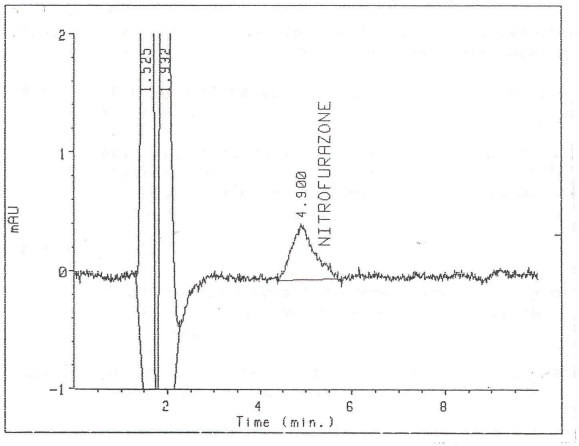


Figure 2.
Detection Limit Chromatogram of Nitrofurazone at 374 nm

2. Sampling Procedure

2.1 Apparatus

2.1.1 A personal sampling pump that can be calibrated to within ± 5% of the recommended flow rate with the sampling device in line.

2.1.2 Glass fiber filters, 37-mm diameter, Gelman Type A or equivalent.

2.1.3 Cassette filter holders for 37-mm filters, Millipore M000037A0 or equivalent.

2.2 Reagents

No sampling reagents are required.

2.3 Sampling technique

2.3.1 Immediately before sampling, remove the plastic plugs from the cassette.

2.3.2 Wrap each cassette with foil to protect it from light exposure.

2.3.3 Attach the cassette to the sampling pump with flexible tubing.

2.3.4 Attach the cassette vertically in the employee's breathing zone in such a manner that it does not impede work performance.

2.3.5 After sampling for the appropriate time, remove the cassette and seal with plastic plugs.

2.3.6 Wrap each sample end-to-end with an OSHA seal (Form 21).

2.3.7 Record the air volume for each sample, and list any possible interferences.

2.3.8 Submit at least one blank for each set of samples. Handle the blank in the same manner as the samples, except no air is drawn through it.

2.3.9 Submit bulk samples for analysis in a separate container. Do not ship with air samples.

2.4 Extraction efficiency

Twenty-four glass fiber filters were each liquid spiked with 10 µL of a 2.34416 mg/mL solution of nitrofurazone. These samples were allowed to dry in the dark at ambient temperature in a drawer overnight. Six of these samples were each desorbed with 5.0 mL of DMF in amber or foil covered vials, shaken for 30 min and then analyzed as in Section 3. The results are listed in Table 2.4.

Table 2.4
Extraction Efficiency

amount spiked, µg	amount found, µg	recovered %
23.442	23.393	99.8
23.442	23.355	99.6
23.442	23.662	100.9
23.442	21.510	91.8
23.442	23.777	101.4
23.442	23.510	100.3
	$\bar{x}$	99.0

2.5 Retention efficiency

The remaining eighteen spiked glass fiber filters from Section 2.4 were placed on a humid air generator and 240 L of humid air (~86% relative humidity) were drawn through each filter at 1 L/min. Six of the filters were each desorbed with 5.0 mL of DMF in amber or foil covered vials, shaken for 30 min and then analyzed as in Section 3. The results are listed in Table 2.5. The remaining samples were stored 6 in a drawer at ambient temperature and 6 in a refrigerator for use in a storage study below.

Table 2.5
Retention Efficiency

amount spiked, µg	amount found, µg	recovered %
23.442	23.262	99.2
23.442	23.606	100.7
23.442	19.928	85.0
23.442	23.364	99.7
23.442	23.359	99.6
23.442	23.510	100.3
	$\bar{x}$	97.4

2.6 Sample storage

After 5 days of storage, 6 samples were each desorbed with 5.0 mL of DMF in amber of foiled covered vials, shaken for 30 min and then analyzed as in Section 3. Three of the samples were from ambient storage and the other three were from the refrigerated storage samples. The remaining samples were analyzed after 8 days of storage. The results are given in Tables 2.6.1 and 2.6.2.

Table 2.6.1
Ambient Storage

amount spiked, µg	amount found, µg	recovered %
23.442	23.064	98.4
23.442	23.093	98.5
23.442	23.660	100.9
23.442	22.456	95.8
23.442	23.049	98.3
23.442	23.362	99.7
	$\bar{x}$ of 5	99.3
	$\bar{x}$ of 8	97.9

Table 2.6.2
Refrigerated Storage

amount spiked, µg	amount found, µg	recovered %
23.442	23.407	99.8
23.442	23.294	99.4
23.442	23.665	101.0
23.442	22.516	96.0
23.442	23.430	99.9
23.442	22.783	97.2
	$\bar{x}$ of 5	100.1
	$\bar{x}$ of 8	97.7

2.7 Recommended air volume and sampling rate

2.7.1 The recommended air volume is 240 L.

2.7.2 The recommended flow rate is 1.0 L/min.

2.8 Interferences (sampling)

It is not known if any compounds will interfere with the collection of nitrofurazone. Any suspected interferences should be reported to the laboratory.

2.9 Safety precautions (sampling)

2.9.1 Attach the sampling equipment in such a manner that it will not interfere with work performance or employee safety.

2.9.2 Follow all safety practices that apply to the work area being sampled.

3. Analytical Procedure

3.1 Apparatus

3.1.1 A balance capable of weighing to the nearest tenth of a milligram. A Mettler HL52 balance was used in this evaluation.

3.1.2 A mechanical shaker.

3.1.3 An HPLC equipped with a UV detector. A Hewlett Packard (HP) 1090M equipped with an autosampler and diode array detector was used in this evaluation.

3.1.4 An HPLC column capable of separating nitrofurazone from any interferences. A 100 mm × 2.1 mm i.d. Hypersil ODS (5 µm) liquid chromatography column was used in this evaluation.

3.1.5 An electronic integrator, or some other suitable means for measuring detector response. The Hewlett-Packard 1090M Data System was used in this evaluation.

3.1.6 Volumetric flasks and pipets.

3.1.7 Vials, 2-mL and 20-mL. (amber or foil covered vials)

3.2. Reagents

3.2.1 Dimethylformamide, HPLC grade, obtained from Burdick and Jackson was used in this evaluation.

3.2.2 Nitrofurazone, reagent grade, obtained from Aldrich (98% purity) was used in this evaluation.

3.2.3 Water, HPLC grade, Milli-Q filtered water, Millipore Inc.

3.2.4 Acetonitrile, HPLC grade, obtained from Burdick and Jackson was used in this evaluation.

3.3 Standard preparation

Prepare nitrofurazone stock standards in subdued light by weighing 10 to 15 mg of nitrofurazone. Transfer the nitrofurazone to separate 10-mL volumetric flasks, and add DMF to the mark. Make working range standards of 2.0 to 185 µg/mL by diluting the stock standards with DMF. Store stock and diluted standards in amber bottles in a freezer.

3.4 Sample preparation

3.4.1 Transfer the glass fiber filter to a 20-mL vial. (amber or foil wrapped vial)

3.4.2 Add 5.0 mL of DMF to each vial and seal with a Teflon-lined cap.

3.4.3 Shake the vials for 30 minutes on a mechanical shaker.

3.4.4 If necessary, transfer the samples to 2-mL amber vials for use on an HP autosampler.

3.5 Analysis

3.5.1 Instrument conditions

Column:	100 mm X 2.1 mm i.d. Hypersil ODS (5 µm)
Mobile phase:	10% acetonitrile 90% water
Flow rate:	0.25 mL/min
Wavelength:	260 and 374 nm
Retention time:	4.7 min
Injection volume:	5.0 µL

3.5.2 Chromatogram:

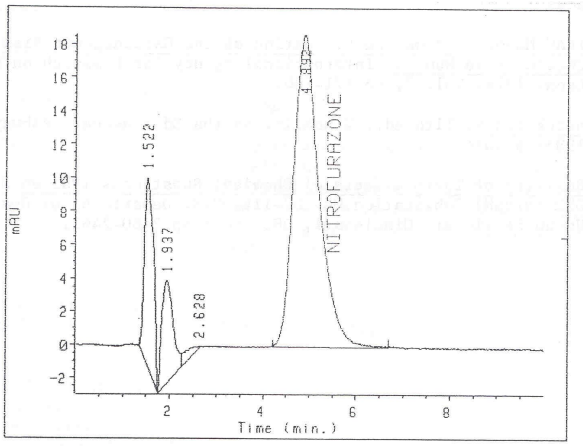


Figure 3.
Chromatogram of Nitrofurazone at 374 nm

3.6 Interferences (analytical)

3.6.1 Any collected compound having a similar retention time to that of the analyte is a potential interference.

3.6.2 HPLC conditions may generally be varied to circumvent interferences.

3.6.3 Retention time on a single column is not proof of chemical identity. Analysis on an alternate HPLC column and confirmation by mass spectrometry are additional means of identification.

3.7 Calculations

3.7.1 Construct a calibration curve by plotting detector response versus concentration (µg/mL) of nitrofurazone.

3.7.2 Determine the µg/mL of nitrofurazone in each sample and blank from the calibration curve.

3.7.3 Blank correct each sample by subtracting the µg/mL found in the blank from the µg/mL found in the sample.

3.7.4 Determine the air concentration by using the following formula.

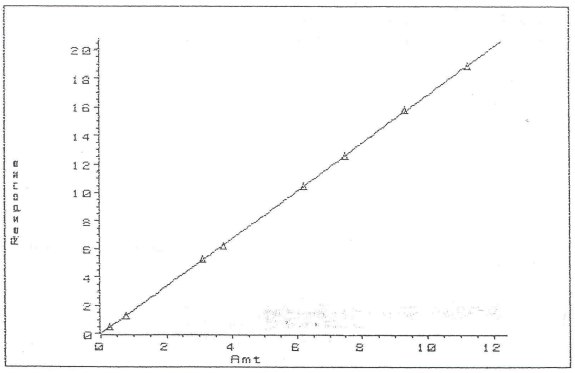


Figure 4.
Calibration Curve

3.8 Safety precautions (analytical)

3.8.1 Avoid skin contact and air exposure to nitrofurazone.

3.8.2 Avoid skin contact with all solvents.

3.8.3 Wear safety glasses at all times.

4. Recommendation for Further Study

4.1 This method should be fully validated.

4.2. Additional information and studies should be obtained on the light sensitivity of nitrofurazone to ascertain the extent of protection necessary. Preliminary studies showed that the response of a nitrofurazone standard in solution would decrease with the duration and intensity of light exposure. Also, it was noted that the recovery of nitrofurazone from spiked filters exposed to light was lower than spiked filters protected from light.

5. References

5.1 IRAC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans; International Agency for Research on Cancer: Lyon, 1974; Vol. 7, pp 171-180.

5.2 Merck Index, 11th ed.; Windholz, Martha Ed.; Merck: Rahway, NJ, 1983; p 1044.

5.3 Registry of Toxic Effects of Chemical Substances 1985-86 Edition; DHHS(NIOSH) Publication No. 87-114, U.S. Department of Health and Human Services: Cincinnati, OH, 1987; pp 2460-2461.