CONTENT OF DIMETHYL FUMARATE IN FOOTWEAR

Chemical Substances and Chemical Preparations

NERI Technical Report no. 819 2011

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Abstract: Dimethyl fumarate (DMF) is used as a biocide preventing moulds that may deteriorate leather furniture or footwear during storage or transport in a humid climate. However DMF may also affects consumers who are in contact with the products because it may cause painful skin contact dermatitis. Pursuant to the EU Commission Decision of 17 March 2009 the Member States have to ensure that products containing the biocide dimethyl fumarate (DMF) are not placed or made available on the market. On behalf of the Danish Environmental Protection Agency (DEPA) the National Environmental Research Institute, Aarhus University, has examined the content of DMF in 302 pieces of footwear on the Danish market in order to comply with the EU Commission Decision. 1 of the 302 examined samples contained DMF in concentrations above the maximum limit of 0.1 mg/kg. The sample contained 0.10-0.17 mg DMF per kg.

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Summary

Dimethyl fumarate (DMF) is used as a biocide preventing moulds that may deteriorate leather furniture or footwear during storage or transport in a humid climate. However DMF may affect consumers who are in contact with the products because it may cause painful skin contact dermatitis.

According to EU Commission Decision of 17 March 2009, the Member States have to ensure that products containing the biocide dimethyl fumarate (DMF) are not placed or made available on the market. On behalf of the Danish Environmental Protection Agency (DEPA), the National Environmental Research Institute, Aarhus University, examined the content of DMF in 302 pieces of footwear on the Danish market, in order to comply with the EU Commission Decision.

1 out of the 302 examined samples contained DMF in concentrations above the maximum limit of 0.1 mg/kg. The sample contained 0.10-0.17 mg DMF per kg.

1 Introduction

Dimethyl fumarate (DMF) is used as a biocide preventing moulds that may deteriorate leather furniture or footwear during storage or transport in a humid climate. However, DMF may also affect consumers who are in contact with the products. DMF penetrates through the clothes onto the consumers' skin where it may cause painful skin contact dermatitis including itching, irritation, redness, and burns. (Rantanen, T., 2008).

Therefore the EU Commission has decided that pursuant to the EU Commission Decision of 17 March 2009 Member States have to ensure that products containing the biocide dimethyl fumarate (DMF) are not placed or made available on the market, and that the presence of DMF in products should be determined against the maximum limit of 0.1 mg DMF per kg of products or part of the product.

The Danish Environmental Protection Agency (DEPA) will examine the content of DMF in footwear on the Danish market in order to comply with the EU Commission Decision.

The present work has been performed as a technical report to DEPA.

2 Samples

Chemical Inspection Service of the Danish Environmental Protection Agency provided 302 samples of footwear for the analysis of DMF. The samples were randomly selected from either whole sale dealers/importers or retailer outlets in Denmark. All the samples were collected in the period August-October 2010 and analysed in the period September-December 2010. The sample with content above 0.1 mg/kg was analysed again in February 2011.

Samples were stored at NERI in plastic bags until the time of analysis. The samples were stored at ambient temperature (approx. 20°C) protected from light.

3 Analytical method

3.1 Principle

The sample is spiked with d_2-labelled DMF and extracted with acetone by using ultrasonic bath. The sample extract is filtered and analysed by Electron Impact Gas Chromatography Mass Spectrometry (EI-GC-MS). The signal for DMF is compared with the signal for d_2-labelled DMF. The analytical method is based on a method described by Narizzano, 2009, but with minor changes e.g. the use of d_2-labelled DMF internal standard.

3.2 Reagents

- Acetone (Rathburne glass destilled)
- Dimethyl fumarate 97% (Fluka)
- Dimethyl fumarate-d_2 98% (CDN Isotopes)
- Naphthalene-d_8 99.9% (Supelco)

3.3 Apparatus

- Filter. 0.45 µm Nylon filter (Phenex, Phenomenex).
- Ultrasonic bath. Branson 5510.
- EI-GC-MS. Agilent 5975C inert XL EI/CI MSD equipped with Agilent 7890A gaschromatograph, split/splitless injector, Agilent autosampler (model 7683B series injector) and Agilent ChemStation software.
- GC analytical column. DB-5ms (J&W) 5% phenylmethyl silicone gum, 30m, 250µm ID, film 0,25µm.
- GC pre-column. VSD (SGE). Deactivated phenylmethyl, 5m, 250/363 µm

3.4 Sample preparation

A part of the arch of the footwear was cut into pieces and mixed. Approximately 2 g was accurately weighed. 0.2 µg d_2-DMF was added (200 µL from 1 µg/ml standard solution). This amount is equivalent to 0.1 mg d_2-DMF per kg sample which is the highest acceptable content of DMF in consumer products. 20 mL acetone was added (or more if a higher amount was necessary to cover the sample). The sample was extracted 20 minutes by using ultrasonic bath and was filtered through glass wool. The extraction was repeated. The extracts were combined and evaporated to less than 1 mL under a stream of nitrogen without heating and transferred to a 1 mL flask. 100 µL injection standard (10 µg/ml d_8-naphthalene solution) was added and the flask was filled up to the mark with acetone. The sample was filtered through a 0.45 µm Nylon filter prior to analysis.

3.5 Analytical conditions

GC

Carrier gas:	helium
Column flow:	1.0 ml/min.
Temperature program:	60 °C in 2 min., 10 °C/min to 320 °C, 320 °C in 10 min.
Equilibration time:	1 min.
Injection volume:	1 µl.
Inlet:	Pulsed splitless, injektionstemperatur 300°C, pressure 14,492 psi, totalflow 54.21 ml/min, septum purge flow 3 ml/min.
Injection pulse pressure:	25 psi to 0,5 min.
Purgeflow to split vent:	50 ml/min in 1 min.

MS

Transferliner, temp.:	280 °C
MS source, temp.:	230 °C
MS quad, temp.:	150 °C
Ionization:	EI mode +70eV
Dimethyl fumarate:	quantification ion m/z 113, qualification ions m/z 85 and m/z 114
Dimethyl fumarate-d_2:	quantification ion m/z 115, qualification ions m/z 87 and m/z 116
Naphthalene-d_8:	quantification ion m/z 136, qualification ions m/z 108

3.6 Screening

The signal of DMF was compared with the signal of d_2-DMF. d_2-DMF was added to the subsamples at the highest acceptable concentration in consumer products (0.1 mg/kg). If the signal of DMF in the subsample was > 50% of the signal of d_2-DMF then a new subsample where screened. Subsamples with signals of DMF > 100% compared with the signal of d_2-DMF were quantified. The injection standard was used to ensure a proper injection by examining the signal visually.

3.7 Quantification

The quantification is based on a calibration curve of 5 concentrations in the range 0.05 - 1 µg/ml, equivalent to 0.025-0.5 mg DMF per kg sample. To estimate the recovery each quantified sample were spiked with 0.1 mg d_2-DMF per kg before sample preparation. The spiked samples were analysed as above. The injection standard was used to ensure a proper injection by examining the signal visually.

3.8 Method validation

Recovery and reproducibility of the method was determined by spiking eight subsamples of a randomly selected sample of the received footwear (sample no. 556) with 0.05 mg d_2-DMF per kg.

Recovery: 76 %, spiked concentration 0.05 mg/kg (n=8)

Reproducibility: 18 % RSD, spiked concentration 0.05 mg/kg (n=8)

Linearity: R^2 = 0.9964 (5 concentrations, range 0.02 - 1 µg/ml equivalent to 0.01-0.5 mg/kg sample.

Table 3.1. Method validation data: recovery and reproducibility.

Sample no.	Method validation data of DMF in footwear		
ATMI-2010-8829-	Spiked concentration	Recovery	RSD
	mg/kg	%	%
556 subsample 1	0,05	86	
556 subsample 2	0,05	78	
556 subsample 3	0,05	102	
556 subsample 4	0,05	67	
556 subsample 5	0,05	82	
556 subsample 6	0,05	67	
556 subsample 7	0,05	66	
556 subsample 8	0,05	62	
Overall	**0,05**	**76**	**18 %**

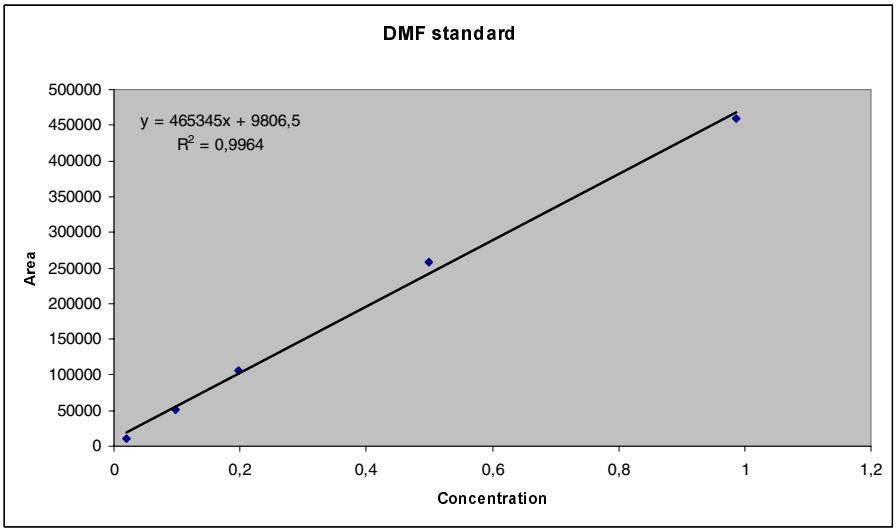


Figure 3.1. Method validation data: Linearity. Range 0.05-1 µg/ml equivalent to 0.025-0.5 mg/kg sample.

4 Results and Discussion

In the present investigation contents of DMF were analysed in 302 samples of footwear. DMF in the samples were identified by comparing GC retention time and mass fragments with the retention times and mass fragments of the standard substance analysed under the same conditions. Performances of the method for quantitative analysis were found to be satisfactory: The recovery at 76% is regarded as satisfactory taking into account the stickiness of the thick evaporated extract and the risk of evaporation of DMF. Due to these reasons, the use of deuterium-labelled DMF as an internal standard is important to ensure correct quantification. The calibration curve of DMF was linear (R^2 = 0.9964) in the investigated concentration ranges.

Figures 4.1-4.2 shows the typical chromatogram of the DMF-standard and the sample containing DMF above 0.1 mg/kg respectively

1 out of 302 examined samples contained DMF in concentrations above 0.1 mg/kg. The signal of DMF was higher than the signal of 0.1 mg d_2-DMF per kg in all analysed subsamples (Table 4.1).

Table 4.1. Content of DMF found in footwear collected from the Danish market.

Sample no. ATMI-2010-8829-	Date of analysis	Content of DMF			
		Content based on external standard	Recovery	Content corrected for recovery	Content based on DMF/d_2-DMF ratio
		mg/kg	%		mg/kg
594subsample 1*)	28/9-10	-	-		0.24
594 subsample 2	1/12-10	**0.17 ±0.03**	77	0.22	0.20
594 subsample 3	14/12-10	**0.12 ±0.02**	81	0.15	0.14
594 subsample 4	16/2-11	**0.10 ±0.02**	87	0.11	0.12
594 subsample 5	16/2-11	**0.11 ±0.02**	82	0.13	0.14
594 subsample 6	16/2-11	**0.13 ±0.02**	62	0.21	0.23
594 subsample 7	16/2-11	**0.11 ±0.02**	72	0.15	0.15

*) The subsample is only analysed with the screening method.

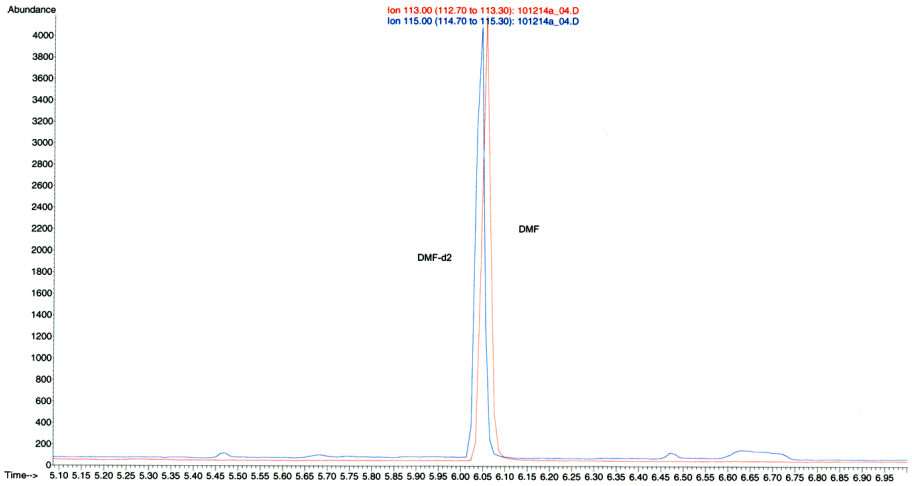


Figure 4.1. Chromatogram of DMF-standard and d_2-DMF-standard. Concentration 0,2 µg/ml equivalent to 0,1 mg/kg sample.

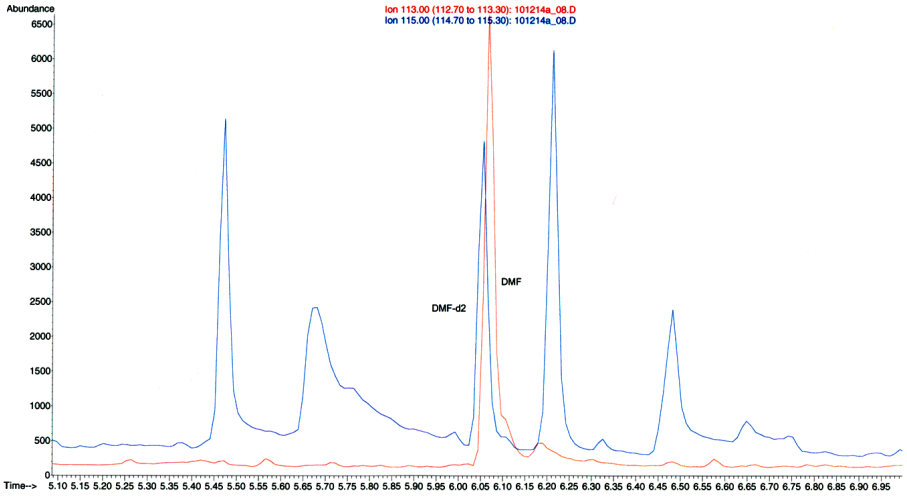


Figure 4.2. Chromatogram of sample no. 594. Concentration of d_2-DMF is 0,2 µg/ml, which is equivalent to 0,1 mg/kg sample.

References

Commission Decision of 17 March 2009 requiring Member States to ensure that products containing the biocide dimethylfumarate are not placed or made available on the market. Official Journal of EEC, No. L74, 20.3.2009, p. 32.

Rantanen,T., 2008. The cause of the Chinese sofa/chair dermatitis epidemic is likely to be contact allergy to dimethylfumarate, a novel potent contact sensitizer. Concise Communication. British Journal of dermatology, no. 159, p. 218-221.

Narizzano, R., et al., 2009: Gas-chromatography – mass spectrometry analysis of dimethyl fumarate in consumer products. Journal of Chromatography A, no. 1216, p. 6762-6766.

Danish version of the report

INDHOLD AF DIMETHYLFUMARAT I FODTØJ

Kemiske stoffer og kemiske produkter

Faglig rapport fra DMU nr. 819 2011

Teddy Krongaard
Kitty K. Petersen
Elsebeth Johansen

DANMARKS MILJØUNDERSØGELSER
AARHUS UNIVERSITET

Sammenfatning

Dimethylfumarat (DMF) anvendes som biocid på lædermøbler og fodtøj for at forhindre produkterne i at mugne under transport og opbevaring i fugtig klima. DMF kan imidlertid også forårsage smertefulde hudallergiske reaktioner for forbrugere, der er i direkte kontakt med de behandlede produkter

Ifølge EU Kommissions beslutning af den 17. marts 2009 skal medlemslandene sikre at produkter, der indeholder dimethylfumarat (DMF) i for høje koncentrationer ikke sælges eller er tilgængelige på markedet. For at efterleve EU kommissionens beslutning har Danmarks Miljøundersøgelser, Aarhus Universitet, for Miljøstyrelsen kontrolleret indholdet af DMF i 302 stykker fodtøj udtaget på det danske marked.

1 ud af de 302 kontrollerede stykker fodtøj indeholdt DMF i koncentrationer over den tilladte grænseværdi på 0,1 mg/kg. Prøven indeholdt 0,10-0,17 mg DMF per kg.

1 Indledning

Dimethylfumarat (DMF) anvendes som biocid på lædermøbler og fodtøj for at forhindre produkterne i at mugne under transport og opbevaring i fugtig klima. DMF kan imidlertid også forårsage smertefulde hudallergiske reaktioner såsom kløe, irritation, rødme og ætsninger på forbrugere, der er i direkte kontakt med de behandlede produkter (Rantanen, T., 2008).

EU Kommissionen har derfor besluttet i EU Kommissionsbeslutning af 17. marts 2009, at medlemslandene skal sikre at produkter på markedet ikke indeholder dimethylfumarat i for høje koncentrationer. Produkter skal derfor kontrolleres for om de indeholder mere end 0,1 mg DMF per kg, der er det maksimalt tilladte indhold i produkter eller dele heraf. Miljøstyrelsen har på baggrund af denne Kommissionsbeslutning valgt at kontrollere indholdet af DMF i fodtøj på det danske marked.

Kontrollen af fodtøj som præsenteres i denne rapport er udført af Danmarks Miljøundersøgelser, Aarhus Universitet (DMU), som teknisk bistand til Miljøstyrelsen.

2 Prøver

Miljøstyrelsens Kemikalieinspektion har indsamlet 302 prøver af fodtøj til analyse for DMF. Prøverne er tilfældigt udvalgt fra enten importører/grossister eller fra forretninger i Danmark. Alle prøver er indsamlet i perioden august – oktober 2010 og analyseret i perioden september - december 2010. Prøven der indeholdt mere end 0,1mg/kg blev analyseret igen i februar 2011.

Prøverne blev modtaget og opbevaret af DMU i plasticposer indtil tidspunktet for analyse. Prøver blev opbevaret ved stuetemperatur (ca. 20°C) og beskyttet mod lys.

3 Analysemetode

3.1 Princip

Prøven er spiked med d_2-mærket DMF og ekstraheret med acetone ved hjælp af ultralyd. Prøveekstraktet er filtreret og derefter analyseret med Elektron Impact Gas Chromatografi Masse Spektrometri (EI-GC-MS). Signalet for DMF er sammenlignet med signalet for d_2-mærket DMF. Metoden er baseret på en metode beskrevet af Narizzano, 2009, men med mindre ændringer, eksempelvis ved anvendelse af d_2-mærket DMF som intern standard.

3.2 Reagenser

- Acetone (Rathburne glass destilled)
- Dimethylfumarat 97% (Fluka)
- Dimethylfumarat-d_2 98% (CDN Isotopes)
- Naphthalen-d_8 99.9% (Supelco)

3.3 Apparater

- Filter. 0.45 µm Nylon filter (Phenex, Phenomenex).
- Ultralydsbad. Branson 5510.
- EI-GC-MS. Agilent 5975C inert XL EI/CI MSD udstyret med Agilent 7890A gaschromatograf, split/splitless injektor, Agilent autosampler (model 7683B series injector) and Agilent ChemStation software.
- GC analyse kolonne. DB-5ms (J&W) 5% phenylmethyl silicone gum, 30m, 250µm ID, film 0,25µm.
- GC for-kolonne. VSD (SGE). De-aktiveret phenylmethyl, 5m, 250/363 µm

3.4 Prøveforberedelse

En del af fodtøjets svang blev klippet i små stykker og blandet. Cirka 2 g blev afvejet præcist. 0,2 µg d_2-DMF blev tilsat (200 µL fra 1 µg/ml standard). Denne mængde svarer til 0,1 mg d_2-DMF per kg prøve som er det højest tilladte indhold af DMF i forbrugerprodukter. 20 ml acetone blev tilsat (eller mere hvis større mængder var nødvendig for at dække prøven). Prøven blev ekstraheret i 20 minutter i ultalydsbad og filtreret gennem glasuld. Ekstraktionen blev gentaget. Ekstrakterne blev kombineret og inddampet til mindre end 1 ml under kvælstof uden opvarmning. Prøven blev overført til 1 ml målekolbe og tilsat 100 µl injektionsstandard (10 ppm naphthalene-d8), hvorefter kolben blev fyldt op til mærket med acetone. Prøven blev herefter filtreret gennem et 0.45 µm nylon filter inden analyse.

3.5 Analyse

GC

Bæregas:	helium
Kolonneflow:	1.0 ml/min.
Temperaturprogram:	60 °C i 2 min., 10 °C/min til 320 °C, 320 °C i 10 min.
Ekvilibreringstid:	1 min.
Injektionsvolumen:	1 µl.
Inlet:	Pulsed splitless, injektionstemperatur 300°C, tryk 14,492 psi, totalflow 54.21 ml/min, septum purge flow 3 ml/min.
Injektionspulstryk:	25 psi til 0,5 min.
Purgeflow til split vent:	50 ml/min i 1 min.

MS

Transferliner, temp.:	280 °C
MS kilde, temp.:	230 °C
MS quad, temp.:	150 °C
Ionisering:	EI mode +70eV
Dimethylfumarat:	kvantificeringsion m/z 113, kvalifikationsioner m/z 85 og m/z 114
Dimethylfumarat-d_2:	kvantificeringsion m/z 115, kvalifikationsioner m/z 87 og m/z 116
Naphthalen-d_8:	kvantificeringsion m/z 136, kvalifikationsion m/z 108

3.6 Screening

Signalet for DMF blev sammenlignet med signalet for d_2-DMF. d_2-DMF blev tilsat prøven i en mængde der svarer til den højest tilladte koncentration af DMF i forbrugerprodukter (0.1 mg/kg). Hvis signalet for DMF i en delprøve var > 50% sammenlignet med signalet for d_2-DMF så blev en ny delprøve screenet. Delprøver med signal for DMF > 100% af signalet for d_2-DMF blev kvantificeret. Injektionsstandarden blev anvendt til at sikre korrekt injektion.

3.7 Kvantificering

Kvantificeringen er baseret på en kalibreringskurve af 5 koncentrationer i intervallet 0,05 - 1 µg/ml, svarende til 0,025-0,5 mg DMF per kg prøve. Genfindingsprocenten er fundet for hver individuel prøve, der er kvantificeret. Dette er gjort ved at tilsætte 0.1 mg d_2-DMF per kg til prøven før prøveforberedelsen. Den spikede prøve blev analyseret som beskrevet ovenfor. Injektionsstandarden blev anvendt til at sikre korrekt injektion.

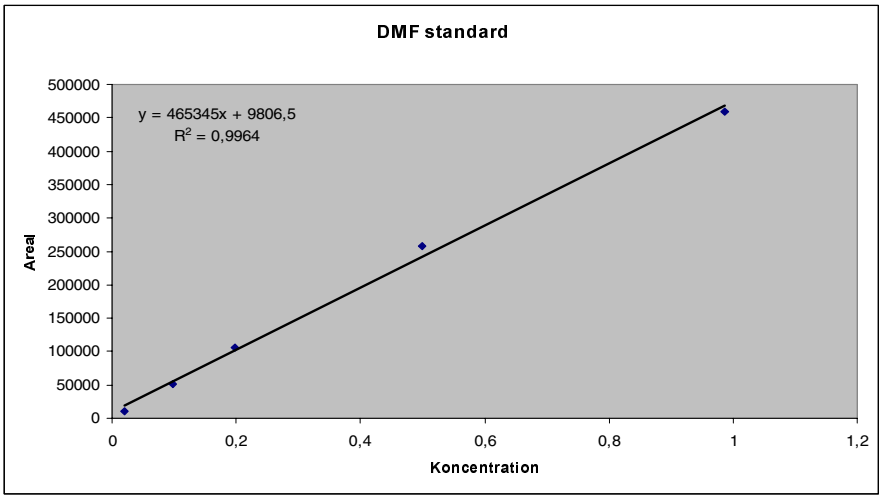
3.8 Metodevalidering

Metodens genfindingsprocent og reproducerbarhed blev bestemt ved at foretage en 8-dobbelt bestemmelse af en tilfældig udvalgt prøve (prøve nr. 556) spiket med 0.05 mg d_2-DMF per kg.

Genfinding:	76 %, spiket koncentration 0,05 mg/kg (n=8)
Reproducerbarhed:	18 % RSD, spiket koncentration 0,05 mg/kg (n=8)
Linearitet:	R^2 = 0,9964 (5 koncentrationer, interval 0,02 - 1 µg/ml svarende til 0,01-0,5 mg/kg prøve.

Tabel 3.2. Data for metodevalidering; genfinding og reproducerbarhed.

Prøve nr. ATMI-2010-8829-	Data for metodevalidering for DMF I fodtøj		
	Spiket koncentration	Genfinding	RSD
	mg/kg	%	%
556 delprøve 1	0,05	86	
556 delprøve 2	0,05	78	
556 delprøve 3	0,05	102	
556 delprøve 4	0,05	67	
556 delprøve 5	0,05	82	
556 delprøve 6	0,05	67	
556 delprøve 7	0,05	66	
556 delprøve 8	0,05	62	
Total	0,05	76	18 %



Figur 3.1. Metodevalideringsdata: Linearitet. Interval 0,05-1 µg/ml svarende til 0,025-0,5 mg/kg prøve.

4 Resultater og Diskussion

I nærværende undersøgelse er indholdet af DMF analyseret i 302 prøver af fodtøj indsamlet fra det danske marked. DMF i prøverne blev identificeret ved at sammenligne GC-retentionstid og massefragmenter med retentionstiden og massefragmenterne af standardmateriale analyseret med samme metode. Metoden til den kvantitative bestemmelse af DMF er fundet acceptabel. Genfindingsprocenten på 76 er tilfredsstillende når det klæbrige ekstrakt og risikoen for fordampning af DMF under inddampningen af prøven tages i betragtning. På grund af disse faktorer er anvendelsen af deuterium-mærket DMF som intern standard vigtig for at sikre korrekt kvantificering. kalibreringskurven for DMF var lineær (R^2 = 0,9964) i det undersøgte koncentrationsinterval.

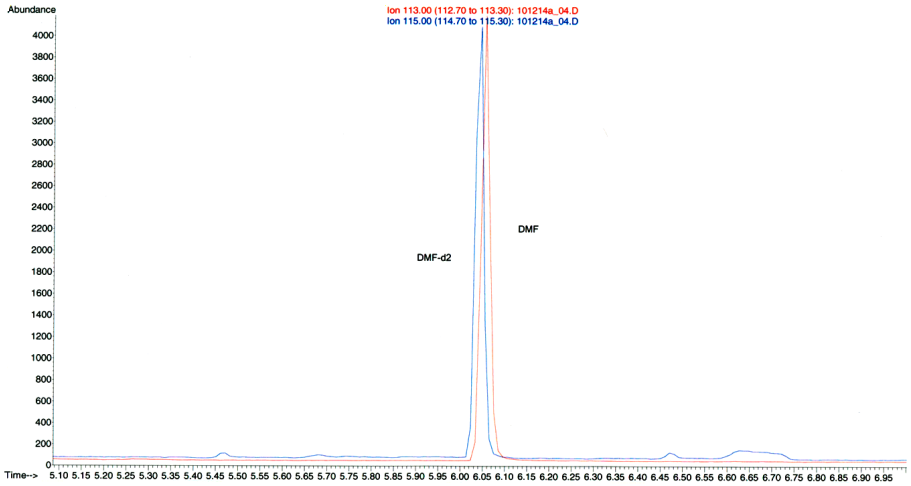
Et typisk kromatogram af DMF-standarderne og prøven indeholdende DMF over 0,1 mg/kg er vist i figur 4.1 - 4.2.

1 ud af 302 undersøgte prøver indeholdt DMF i koncentrationer over 0,1 mg/kg. Signalet for DMF var højere end signalet for 0,1 mg d_2-DMF per kg i alle analyserede delprøver (Tabel 4.1).

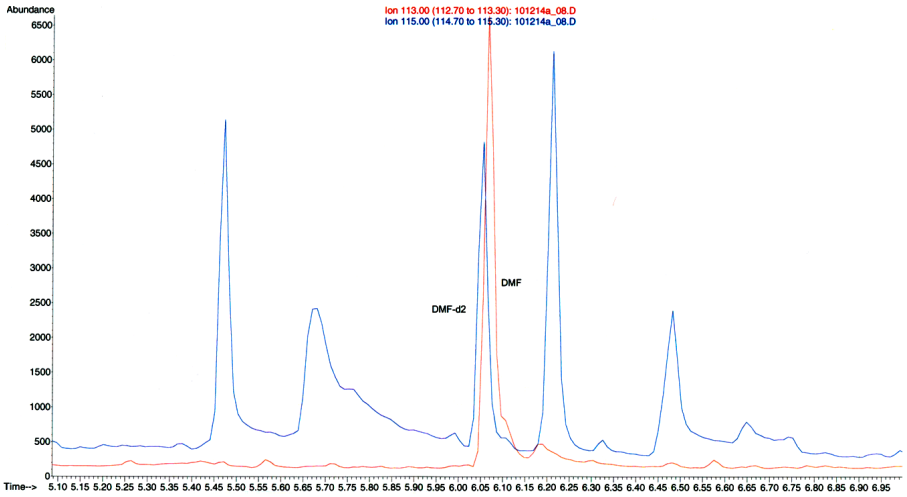
Tabel 4.1. Indhold af DMF fundet i fodtøj indsamlet på det danske marked.

Prøve nr. ATMI-2010-8829-	Dato for Analyse	Indhold af DMF			
		Indhold baseret på ekstern standard	Genfinding	Indhold korrigeret for genfinding	Indhold baseret på DMF/d_2-DMF forhold
		mg/kg	%		mg/kg
594 delprøve 1[*)]	28/9-10	-	-		0.24
594 delprøve 2	1/12-10	**0.17 ±0.03**	77	0.22	0.20
594 delprøve 3	14/12-10	**0.12 ±0.02**	81	0.15	0.14
594 delprøve 4	16/2-11	**0.10 ±0.02**	87	0.11	0.12
594 delprøve 5	16/2-11	**0.11 ±0.02**	82	0.13	0.14
594 delprøve 6	16/2-11	**0.13 ±0.02**	62	0.21	0.23
594 delprøve 7	16/2-11	**0.11 ±0.02**	72	0.15	0.15

[*)] Prøven er kun analyseret med screeningsmetoden.



Figur 4.1. Kromatogram af DMF-standard og d_2-DMF. Koncentration 0,2 µg/ml svarende til 0,1 mg/kg prøve.



Figur 4.2. Kromatogram af prøve nr. 594. Koncentration af d_2-DMF er 0,2 µg/ml svarende til 0,1 mg/kg prøve.

References

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CONTENT OF DIMETHYL FUMARATE IN FOOTWEAR

Chemical Substances and Chemical Preparations

Dimethyl fumarate (DMF) is used as a biocide preventing moulds that may deteriorate leather furniture or footwear during storage or transport in a humid climate. However DMF may also affects consumers who are in contact with the products because it may cause painful skin contact dermatitis. Pursuant to the EU Commission Decision of 17 March 2009 the Member States have to ensure that products containing the biocide dimethyl fumarate (DMF) are not placed or made available on the market. On behalf of the Danish Environmental Protection Agency (DEPA) the National Environmental Research Institute, Aarhus University, has examined the content of DMF in 302 pieces of footwear on the Danish market in order to comply with the EU Commission Decision. 2 of the 302 examined samples contained DMF in concentrations above the maximum limit of 0.1 mg/kg. These two samples contained 0.13-0.23 mg DMF per kg.

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